PREDICTING FORAGE NUTRITIVE VALUE USING AN IN VITRO GAS PRODUCTION TECHNIQUE AND DRY MATTER INTAKE OF GRAZING ANIMALS USING N-ALKANES

A Thesis

by

ANDRÉ DE STEFANI AGUIAR

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

May 2010

Major Subject: Animal Science

PREDICTING FORAGE NUTRITIVE VALUE USING AN IN VITRO GAS PRODUCTION TECHNIQUE AND DRY MATTER INTAKE OF GRAZING ANIMALS USING N-ALKANES

A Thesis

by

ANDRÉ DE STEFANI AGUIAR

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

Approved by:

Co-Chairs of Committee,	Luis O. Tedeschi
	F. Monte Rouquette, Jr.
Committee Members,	Thomas D. A. Forbes
	Ronald Randel
Head of Department,	Gary Acuff

May 2010

Major Subject: Animal Science

ABSTRACT

Predicting Forage Nutritive Value Using an In Vitro Gas Production Technique and Dry Matter Intake of Grazing Animals Using n-Alkanes.

(May 2010)

André De Stefani Aguiar, B.S., Faculdades Associadas de Uberaba

Co-Chairs of Advisory Committee: Dr. Luis Tedeschi
Dr. Monte Rouquette

In the first experiment, forage samples (n = 39) were collected during 4 years (2006 – 2009) from pastures grazed by Santa Gertrudis cattle at the King Ranch, TX. The in vitro gas production technique (IVGP) was performed to understand the pattern of fermentation parameters of the forage and obtain fractional digestion rate (kd) values to predict total digestible nutrients (TDN). The best nonlinear model to describe the IVGP values of the forages was the two-pool logistic equation. The passage rate (kp) of 4%/h was used.. The kp predicted by the Large Nutrient Ruminant System (LNRS) model was 3.66%/h. The average TDN was 55.9% compared to 53.8% using a theoretical equation. In the second experiment, Brahman bulls (n = 16) grazed Coastal bermudagrass pastures [*Cynodon dactylon* (L.) Pers.] and stocked at a moderate to low grazing pressure. Three periods of fecal collections were made within each period. Bulls were individually fed at 0700 and 1900 h of 400 g of corn gluten pellets containing C_{32} n-alkanes. Each period was divided in 2 sub periods in which

fecal samples were collected 4 times a day (0700, 1100, 1500 and 1900 h). N-alkanes in the forage and feces were determined using gas chromatography. In the third experiment, four methods were used to estimate dry matter intake (DMI): C_{31} or C_{33} with or without adjustment for forage C_{32} (C_{31_0} and C_{33_0}, respectively). There was a difference between morning (0700 and 1100 h) and afternoon fecal collections (1500 and 1900 h) on the predicted DMI using C_{31} ($P = 0.0010$), C_{33} ($P = 0.0001$), C_{31_0} ($P = 0.0010$), or C_{33_0} ($P < 0.0001$). There was no difference in average daily gain (ADG) between low and high residual feed intake (RFI) ($P = 0.5709$). The nonparametric analysis indicated that pre-ranking animals for efficiency under confinement conditions does not guarantee ($P < 0.0001$) similar ranking under grazing conditions when using the alkane technique to determine forage DMI. In order to estimate DMI at least 5 d of fecal collection and 2 times a day of collection (0700 and 1500h) are needed to decrease the variability.

To my family

ACKNOWLEDGMENTS

First of all, I would like to thank God and Nossa Senhora de Aparecida for the protection and blessing; to my parents, Heliodoro Henrique Aguiar and Renata Maria De-Stefani Aguiar, who have worked very hard towards my personal and professional life, and my sister, Priscilla De-Stefani Aguiar, and Robson Palermo, for their support and confidence. I love you all with all of my heart.

To my nonos and my grandparents Dino De-Stefani e Brígida Amália Rosalia Brodela De-Stefani, Dirceu Aguiar e Doraci De Mateo Aguiar, for their encouragement and support.

I also would like to thank Dr. Tedeschi and Dr. Rouquette for giving me the opportunity to gain the knowledge base and experience necessary to accomplish my goals. I am forever in your debt and certainly owe my entire future to your teaching, ethics, and sincerity. I also thank my committee members, Dr. Randel, and Dr. Forbes, for readily accepting to participate in my committee and for their helpful suggestions and assistance. I also would like to thank Mr. Joel Kerby, Mr. Gary Nimr, and Mrs. Carrie Hensarling, for helping me during my data collection and data analysis. I also would like to thank Dr. Ortega and Dr. McCuistion for their help with the forage collections, and Dr. Anderson for allowing me to use the USDA cows to collect ruminal fluid.

Thanks to all my friends, especially Dr. Flávio Ribeiro, Dr. Mario Chizzotti, Dr. Douglas Pina, Marcos Marcondes, and all the exchange students that helped me during the laboratory analyses. Thanks to Mr. Pat Cone and his family for the support in the USA.

TABLE OF CONTENTS

Page

LIST OF TABLES

TABLE Page

LIST OF FIGURES

CHAPTER I

INTRODUCTION

The United States beef industry has more than 960,000 ranches with more than 74 million head of cattle (USDA, 2007). In addition, $65 billion was added in 2007 by producers of meat animals to the US economy (USDA, 2008). Beef production has gone through major changes from 2006-2009 due to increased utilization of corn grain by ethanol plants; and increases in the prices of fertilizer and feedstuffs, and corn per se have impacted the sector. The use of grazing systems to background calves for a longer period of time, or even to finish them, is increasing largely due to changes in the price of corn (Quanbek and Johson, 2009). A major change is that ranchers are maintaining animals under grazing conditions for a longer period of time; in turn, beef production from pasture systems has grown in the past few years (Rouquette et al, 2009). Corn prices reached $0.29/kg ($7.34/bushel) in August (CBOT, 2008) and that increase has led to modifications in feed management practices of livestock producers in the US. Although the price declined to $0.18/kg ($4.00/bushel), there is still competition for corn between ethanol plants and the animal industry. The landscape across the US has enough pastures, forages, and grasslands to sustain ruminant production year around (Burns, 2008).

In forage forage-based systems the quality of the forage is essential for the success of the system; quality can be divided into chemical composition and nutritive value (Gizzi and Givens, 2004). Forage quality is described as the capacity of the forage to meet animal re-

---

This thesis follows the style and form of the Journal of Animal Science.

quirements, and it is the first limiting issue in ruminant productivity (Collins and Ortiz, 2003). There are several methods to measure chemical composition; the most common are wet chemical analysis and near infrared reflectance spectroscopy. The nutritive value of the feed can be achieved using digestibility techniques, in vivo, in vitro, and the end products of digestion (Collins and Ortiz, 2003). In the past, the in vivo technique was used more frequently until McBee (1953) developed an in vitro gas production system (**IVGP**). Since then, this technique has been used and improved to predict nutritive values of feedstuffs. The benefits of IVGP include, being less invasive than in situ techniques and the opportunity to estimate digestibility, fermentation kinetics, and VFA profiles. Tedeschi et al. (2008b) reported that IVGP has been frequently used to assess nutritive values of feeds based on their pattern of accumulated gas when incubated with rumen fluid under anaerobic conditions.

According to Minson (1990), one of the most important aspects in ruminant production under grazing systems is the quantity of the forage consumed. Dove and Mayes (1996) stated that measure what animals are eating, the quality and the quantity, when, and where they are consuming the forage is required to study the feeding and behavior, and nutrition of mammalian herbivores. According to Minson (1990), the available forage mass and forage quality have an effect on the DMI of grazing animals. Welch and Hooper (1988) enumerated factors affecting DMI to be forage quantity, palatability, environmental conditions, social effects, and physiological status of the animal. Using the alkane technique to predict DMI was firstly proposed by Mayes and Lamb (1984). They pointed out the possibility of using n-alkanes (plant waxes) as an indigestible marker, and they concluded

that n-alkanes were more chemically inert and simple to analyze than long-chain fatty acids. The alkane technique has been used to predict DMI in grazing animals (Mayes and Lamb, 1984; Dove and Mayes, 1991; Bovolenta et al., 1994; Hameleers and Mayers, 1998; Oliván et al., 2007).

The profitability of livestock production in grazing systems is related to the efficiency of converting forage into products, the quantity and quality of forage produced, and the ability of producers to manage the forage (Forbes, 1988). The knowledge of the amount of forage being consumed by grazing animals is important because it is the major cost input in most animal production systems (Herd et al., 2003). The conversion of feed into body tissues differs between animals and it is commonly measured as feed efficiency. Golden et al. (2008) also pointed out that improvements have been made in diet formulation models; however, a difference between individuals in a phenotypic expression of efficiency still exists. Feed efficiency is not a direct measurable trait because it depends on measurements of DMI (Koch et al., 1963). Reducing inputs (feed DMI) is necessary to improve profit in animal production (Herd et al, 2003). The technique that has been used to identify efficient animals is residual feed intake (**RFI**). Feed efficiency is a combination of intake and performance (Herd et al, 2003). The animals are separated into efficient (low RFI) and inefficient (high RFI) groups. Efficient animals eat less than expected but have the same ADG and mean metabolic body weight of inefficient ones. Therefore, for grazing animals, reliable estimates of DMI and forage quality are needed to accurately determine RFI; and to compare the impact of pre-selected RFI animals and the animals' efficiency when under grazing conditions.

Objectives

The first objective was to investigate the pattern of in vitro fermentation parameters of forages obtained at the King Ranch in South Texas throughout four consecutive years (2006, 2007, 2008, and 2009). Outcomes of this objective will provide information on seasonal differences in forage quality, and will provide information needed to formulate supplemental feeds (energy and/or protein) whenever required. Another goal was to develop an equation to calculate TDN based on IVGP measurements and chemical analysis of the forages.

The second objective was to identify factors that contribute to the variation in the DMI of grazing cattle using the n-alkane technique and to determine the optimum fecal collection period for DMI determination of cattle grazing Coastal bermudagrass using the n-alkane technique.

The third objective was to compare the RFI of animals determined under confinement conditions with the RFI ranking of the same animals when fed under grazing conditions. The forage DMI was determined using the alkane technique as detailed in the second objective.

CHAPTER II

LITERATURE REVIEW

In 2000, the world population was around 6 billion people. Today it is about 7 billion and the forecast for 2050 is 9.5 billion people (United Nations, 2004). The demand for food has increased almost 30% (milk and beef) in the last five years and the demand for food crops has also increased (Makkar, 2004). Corn price in 2009 rose 49% compared to 2006. Corn production in the 2006/2007 season in the United States was 266.700 tons. The ethanol production in the United States consumed 25% of the corn harvest (NCGA, 2008) whereas livestock and poultry industries consumed about 55% of the corn produced (Leibtaq, 2008). Ethanol production is competing with the livestock industry for corn grain utilization. Therefore, it becomes necessary for innovative and different strategies for ruminant animal production. Animal productivity per acre has to increase to supply the demand for food while maintaining a sustainable environment. According to the USDA (2008), 317 million hectares were used in 2002 for grazing systems in the United States, and in 1997 around 57 million animal units (60% of the herd) grazed forage lands. Van Soest (1994) stated that ruminants are able to digest and have fiber material as their diet due to the anatomical design and physiological functional of their gastrointestinal system.

Management in the beef industry is slowly changing. In the past, many producers used to send their animals to feedlot earlier (6-10 months of age). Today, many are retaining their cattle on pastures until 10-15 months of age to decrease production cost and increase profit potential. Animal production on pasture is extremely important because of the ability to buffer the total production cost of finished animals. Most of the cow/calf production

systems in the US are done under grazing conditions (Rouquette, 2009). Therefore, the ability to produce sufficient forage with adequate quality is important for the overall system. In livestock production, maintaining feed quantity and quality available throughout the year is the major management challenges. Feed analyses are required to monitor the quality of the forage to allow producers to use the information to make decisions regarding supplementation and animal management strategies throughout the year. In a beef cow-calf system, feed and hay account for nearly 60% of the production cost in the US (USDA, 2005).

Determining Forage Quality

Because forages are the main source of feeds for ruminants under grazing systems (Burns, 2008), maintenance of forage quality and availability are crucial for successful beef production. Ellis et al. (1999) defined nutritive value as the chemical contents and their transformation to nutrients to meet animal requirements. Coleman et al. (1999) suggested that feedstuff analysis for ration nutrient balancing and interactions between feeds and animals are required to increase the productivity of beef production and to achieve the producer's goals. According to Nocek (1988), there are numerous techniques to estimate digestibility either using laboratory and/or animal methodology. Digestibility is the fraction of a feedstuff or dietary feeds that is digested and absorbed while passing through the digestive tract (Cochran and Galyean, 1995). Digestibility is calculated as the total of amount forage or feed consumed minus total amount of feces excreted divided by the

amount of DM consumed. There are numerous factors that can interfere with feedstuffs digestibility, including physiological age of the forage, chronological age, season of the year, level of intake (Maynard et al., 1979), environmental factors (NRC, 1981), forage cell wall concentration and constituents (Van Soest, 1965), and passage rate (Sniffen et al., 1992). Van Keuren and Heinemann (1962) described the in situ DM digestibility of alfalfa hay using nylon bags and concluded that particle size and diet may also affect digestibility.

One of the methods most widely used to measure digestibility in ruminants during the last decade is the in vivo fermentation technique. Methods used to evaluate feedstuffs utilizing in vivo techniques are expensive. These require a large amount of labor, large amounts of feedstuffs, are time consuming, and require expensive facilities (Adesogan, 2005). According to Barnes (1968), laboratory methods for the evaluation of quality have to be based on in vivo data.

The in vitro gas production (**IVGP**) technique was initially developed by McBee (1953) and later refined by Hungate (1966). Later, Tilley and Terry (1963) proposed the two-stage methodology to estimate in vitro digestibility and to decrease the amount of feed necessary to evaluate feedstuffs. The IVGP technique was developed to predict fermentation of ruminant feedstuffs (Rymer, 2005). Researchers from different parts of the world and from different fields have been using IVGP due to the possibility to study the impact of livestock production on the environment (Krishanmoorthy et al. 2005). According to Dahnoa et al. (2004), feed evaluation and studies involving ruminal fermentation have used the in vitro fermentation technique. This technique yields reliable measurements of rates of fermentation of fiber that can be used to determine energy availability of feeds. The

precision of the results is a product of the accuracy and reproducibility of the method (Tilley and Terry, 1963). The errors are cumulative. Therefore, in a method like that a mistake at the start of the process can change the result of the research. According to Getachew et al. (2004), ruminant productivity is related to the accuracy of analysis of the quality and composition of forage and meals. Although traditional in vitro methods measure the digestibility of one substrate component, IVGP techniques measure the fermentation of insoluble and insoluble substrates (Tilley and Terry, 1963).

One of the advantages of the IVGP technique is that it can be automated to generate a larger number of data points which allow for more accurate parameter estimation than gravimetric in vitro techniques or in situ methods (Huhtanen et al., 2008). Other benefits of the IVGP technique include: being a less invasive technique than in situ techniques, and being able to estimate digestibility, fermentation kinetics, and volatile fat acid profiles concurrently. The IVGP technique can measure the impact of feedstuffs on methane production, as well as the effect of methane inhibitors. The IVGP technique allows for the study of effects of feed additives and supplements on digestibility, and for evaluating of microbial activity. Currently, the energy value of the feeds is computed by summing the values without considering the interactions between the ingredients, where one nutrient can alter the availability of another nutrient (Getachew et al., 2005). France et al. (2005) reported that IVGP can measure the rate of degradation of feedstuffs and the accumulated gas profile varies according to the shape and the slope of the fermentation curve. They also pointed out that during the early stages of the fermentation, the slope has a tendency to be zero, and a sigmoidal curve starts to be produced as the degradation of the substrate commences. Van

Soest (1991) stated that even though systems of chemical analyses are fast and accurate, they do not reflect the biological reality that can be reached with in vitro systems. The knowledge of pool size and digestion rate of the NDF part of the forage and concentrate are another advantage of the IVGP technique (Schofield and Pell, 1995).

Tilley and Terry (1993), working with grasses, reported a good relationship of digestibility between predicted data (in vivo) and observed data (in vitro) with a linear regression equation ($Y = 0.99 \times X - 1.01$, SE = ±2.31). The digestibility estimate by the IVGP was highly correlated with that predicted by in vivo methods (Marten and Barnes, 1980). Monson et al. (1968) reported a high correlation ($r = 0.92$) between digestibility in vivo and in vitro of Coastal bermudagrass. A significant relationship ($r = 0.79$) between in vitro and in vivo digestibility was found by Sileshi et al. (1996). Blummen et al. (1997) working with roughages reported a high correlation between IVGP and apparent and true digestibility ($r = 0.96$ and 0.95; respectively). However, Varel and Kreikemeier (1995) reported a difference in lag time, shorter for in vivo; faster digestion rate for in vivo; and greater extent of digestion for the in vivo technique. A difference in digestibility was found by Monson and Reid (1968). They suggested the difference was due to an inappropriate sampling of the forages.

Determining Forage Consumption

After knowing the quality of the forage and the herbage mass of the grazed paddock, the next step is to estimate forage consumption. This task has been a challenge for decades.

The measurement of DMI is vital to evaluate the nutritional status and expected performance of animals in all production systems (Ferreira et al., 2004). For the last several decades, one of the most common marker methods to determine DMI was based on chromic oxide (Cr_2O_3) as an external marker. The Cr_2O_3 is used as an external marker either using gelatin capsules or adding it into a paper (Dove and Mayes, 1995). Herd et al. (2004), working with controlled-release devices to predict DMI in grazing steers, reported a malfunction of the method in 10% of the animals. The marker technique has some errors due to the recovery of the marker, the use of a single value for digestibility for the forage, and it is an unpleasant material to handle (Malossini et al., 1996).

The use of n-alkanes as an indigestible fecal marker was first proposed by Mayes and Lamb (1984). Since then, several researchers have agreed that the n-alkane technique is extremely useful to estimate DMI and digestibility concurrently (Mayes et al., 1986; Bovolenta et al., 1994; Mayes et al., 1995; Lippke, 2002; Martins; 2002; Fushai, 2006; Oliván et al., 2007). An ideal marker cannot be digested in the gastrointestinal tract; it should pass at the same rate as the marked digesta, and must not affect or be affected by the gastrointestinal tract and/or microbial population (Dove and Mayes, 1991; Giráldez, 2006). Mayes and Lamb (1984) pointed out the possibility of using n-alkanes (waxes) as an indigestible marker and they concluded that n-alkanes are more chemically inert and simple to analyze than long-chain fatty acids. Vulich et al. (1995) pointed out that in order to determine DMI the methodology requires the determination of the ratio of n-alkanes in the feces.

The profitability of livestock production in grazing systems is related to the efficiency of converting forage into products and the quantity and quality of forage produced, and the ability of the producer to manage the forage (Forbes, 1988). According to Dove and Mayes (2006), measurement of what animals are eating, diet quality and quantity, and consumption patterns of the diet are required to study the feeding and nutritional behavior of mammalian herbivores. Lippke (2002) suggested that there is no practical way to measure DMI by grazing animals directly; it has to be done by measuring or estimating total fecal output and the digestibility of the diet. The n-alkanes technique has been used as a viable technique to predict DMI in grazing animals (Mayes and Lamb, 1984; Dove and Mayes, 1991; Bovolenta et al., 1994; Hameleers and Mayers, 1998; Oliván et al., 2007). The knowledge of the amount of forage being consumed by grazing animals is important because it is the major cost input in most animal production systems (Herd et al., 2003).

Currently, the marker that has been widely used to predict DMI is hydrocarbons of plant cuticular wax (odd-chain n-alkanes) dosed with even-chain n-alkanes (Bovolenta et al. 1994; Hameleers and Mayers, 1998). Dosed alkanes have higher recovery compared to naturally occurring alkanes (Mayes et al., 1986). The recovery of fecal n-alkanes increases as carbon chain length increases (Mayes et al. 1986; Bovolenta et al., 1994). The n-alkanes (waxes) present in herbage species are odd-chain (C_{25} – C_{35}). Hence, the most abundant n-alkanes that are present in the herbage are C_{29}, C_{31}, and C_{33} (Mayes et al., 1984). Several authors (Mayes et al., 1986; Bovolenta et al., 1994; and Dove and Mayes, 2006) have pointed out that octacosane (C_{28}) and dotriacontane (C_{32}) can be used as external markers due to their simple and cheap access and their low concentration in forages. These authors

also concluded that alkanes can be used to estimate DMI without using total fecal collection. According to Vulich et al. (1991), the alkane technique does not require an independent estimate of digestibility, nor the measurable recovery of n-alkane dosed in order to predict DMI. Malossini et al. (1996) concluded that increasing the number of fecal samplings allows for decrease of measurement errors.

In contrast, Dove and Mayes (1991) pointed out that total fecal collection can affect grazing behavior, and requires greater amounts of labor and is unpleasant to do. Mayes et al. (1986) reported an incomplete recovery for alkanes, and ruminants may utilize alkanes present in the diet. They also pointed out that scientists do not really know the behavior of the alkane in the digestive tract or if the concentration of the alkane in the forage has an influence on fecal recovery. Another problem brought up by different authors is the diurnal variation in the fecal excretion of alkanes (Mayes et al., 1986; Vulich and Hanrahan, 1995, Oliván, 2007) if animals are not dosed twice a day.

Residual Feed Intake on Grazing Animals

Recently, researchers have studied improvements related to efficiency of gain (**G:F**) of animal production (Golden et al., 2008). Golden et al. (2008) indicated that improvements have been made in diet formulation models; however, a difference between individual animals in phenotypic expression of efficiency still exists. Lancaster et al. (2009) pointed out that selecting animals for feed efficiency is a method that can be used to improve profit in beef production. There is variation in DMI between individuals. The difference between the

animals expected consumption and the amount consumed, based on average growth rate and average metabolic BW, is referred to as residual feed intake (**RFI**). Herd and Author (2009) suggested that RFI is a phenotypically independent trait; and it can be used to estimate DMI and make comparison between individuals. The RFI was firstly proposed by Koch et al. (1963) in order to select animals for feed efficiency. According to Herd et al. (2004), metabolic mean BW and ADG are not correlated with RFI, as expected from the linear regression.

Animals that have a negative RFI are more efficient than those with a positive RFI. Animals with a negative RFI eat less feed but still have the same performance as the positive ones. This can decrease the amount of feed required, and consequently this selection may decrease production cost and improve profitability (Arthur et al. 2004). Dobos and Herd (2008) suggested that RFI may be used to select animals that consume less feed with no reduction in performance. To determine RFI values for individual animals, feed intake, and performance must be quantified. Golden et al. (2008) suggested that more research is needed to understand the patterns of intake and the relationship to feed efficiency. Lancaster et al. (2009) concluded that RFI can be used to select animals for feed efficiency.

Although there have been extensive experiments using confined feeding technology, there is a lack of RFI data under grazing conditions. Measurement of intake in grazing systems is more difficult as it is necessary to predict of feed consumed. Some research has shown that animal RFI rankings in confined feeding regimen will maintain the same ranking when fed under grazing conditions (Herd et al., 2003). However, Meyer et al. (2008) reported no differences in intake between low and high RFI cows, during gestation and late

lactation on pasture. Those results concur with Herd et al. (1998), who worked with pre-ranked cows nursing calves under feedlot conditions, and found no difference in DMI under grazing conditions. Finally, due to the lack of research using grazing animals to measure RFI, and DMI, our objectives were to compare RFI of animals determined under confinement conditions with RFI determined under grazing conditions.

CHAPTER III

DETERMINATION OF NUTRITIVE VALUE OF FORAGES IN SOUTH TEXAS USING AN IN VITRO GAS PRODUCTION TECHNIQUE TO ESTIMATE TOTAL DIGESTIBLE NUTRIENTS

Overview

Animal production under grazing systems requires reliable and rapid forage analysis for appropriate management such as supplementation strategies and stocking rate. The objectives of this study were to investigate the use of the in vitro gas production (**IVGP**) technique to understand the pattern of fermentation parameters of forages obtained from pastures in the South Texas; to obtain empirical relationships between the IVGP fermentation parameters and chemical composition of the forages, and to use the IVGP data to develop equations to predict total digestible nutrients (**TDN**). During 4 consecutive years (2006 – 2009), forage samples were collected monthly (n = 39) at the King Ranch from pastures grazed by Santa Gertrudis cows. Adequate climatic, animal, and feed information were inputted in the Large Ruminant Nutrition System (**LRNS**, v. 1.01) to predict energy and protein balances monthly throughout the study period. The IVGP data was fitted to nonlinear models using the Gas Production Fitting System v. 2.3 with the best nonlinear model to describe the IVGP values of the forages being the two-pool logistic equation. For 2006, 2007, 2008, and 2009, the average lag times, h, were 6.47 ± 0.54; 7.75 ± 0.65; 7.49 ± 2.01; and 5.44 ± 1.46, respectively. The average ratio of ml of gas per mg of DM was 0.41 ± 0.11, 0.34 ± 0.09, 0.34 ± 0.07, and 0.26 ±0.10 for 2006, 2007, 2008, and 2009, respectively, suggesting a consistent decline in the nutritive value of the forage. There was a moderate

negative correlation (r = -0.53) between lignin and NDF, and a moderate positive correlation (r = 0.58) between CP and NDF digestibility. The average values for TDN at the maintenance DMI level (**TDN_{1x}**) and without adjustment for unavailable carbohydrate were 56 ± 5.09, 49 ± 5.22, and 45 ± 5.28% for passage rates of 4, 6, and 8 h^{-1}; respectively. The TDN_{1x} computed by a theoretical equation was 53.8 ± 3.44%. Based on our evaluations, a kp of 4 %/h would reflect the typical forage passage rate for these beef cows grazing low quality forage. The average TDN for kp of 4%/h was 55.9%. The predicted kp by the LRNS model using the level 2 solution was on average 3.66 %/h. This value supports our assumptions of using a kp of 4 %/h. Our results suggested that several climatic factors may affect fiber digestibility. We concluded that IVGP data can be used in predicting TDN values in warm-season forages.

Introduction

In a cow/calf system, forage is the major source of energy and protein for the animals. Increases in the duration of grazing period and decreases in the amount of supplementation for beef cows are alternatives to decrease production costs and to increase the potential for profitability. Feed and hay are the major costs in cattle production (Quanbek and Johson, 2009). The main challenge in producing cattle under grazing systems is to maintain forage nutritive value and DM mass available throughout the year. Therefore, reliable and more rapid forage analyses are needed to accurately determine the availability of energy and nutrients of the forage.

Allen and Segarra (2001) reported that forage quality is best described as the degree to which forage meets the nutritional requirements of a specific kind and class of animal. According to Adesogan (2005), evaluation of feeds requires expensive facilities, large amounts of time, and labor. Dahnoa et al (2004) suggested that feed evaluation and studies involving ruminal fermentation have used in vitro fermentation techniques. The in vitro gas production technique (**IVGP**) was initially developed by McBee (1953) and later refined by Hungate (1966). Tilley and Terry (1963) proposed a two-stage methodology to estimate in vitro digestibility and to decrease the amount of feedstuff necessary in the original method. The IVGP technique was developed to predict fermentation of ruminant feedstuffs (Rymer et al., 2005). The IVGP technique has been used to evaluate forages because the fermentation kinetics allow for an evaluation of distinct phases of gas production; therefore, the soluble and insoluble fractions of the forage can be evaluated separately (Makkar, 2004).

One of the advantages of the IVGP technique is that it can be automated to obtain a large number of data points allowing for a more accurate determination of parameter estimates than the gravimetric in vitro techniques or in situ methods (Huhtanen et al., 2008). Other benefits of the IVGP technique include being less invasive than in situ techniques, and allows for estimates of digestibility, fermentation kinetics, and VFA profiles. Tedeschi et al. (2009) reported the IVGP technique has been frequently used to assess nutritive values of feeds based on their pattern of accumulated gas when incubated with rumen fluid under anaerobic conditions. Therefore, the combination of chemical analyses and the IVGP technique might yield reliable measurements of rates of fermentation of fiber that can be used to determine energy availability of feeds for ruminant animals.

The objectives of this study were: (1) to use the IVGP technique to study the pattern of in vitro fermentation parameters of forages obtained from pastures throughout the year in South Texas at the King Ranch during four consecutive years (2006, 2007, 2008, and 2009); (2) to obtain empirical relationships between the IVGP technique fermentation parameters and chemical composition of the forages; (3) to develop equations to compute TDN; and (4) to perform simulations to predict ME and MP balance in Santa Gertrudis cows.

Material and Methods

Forage samples (most dominant species were Kleberg bluestem [*Dichanthium annulatum*] and Coastal bermudagrass [*Cynodon dactylon* (L.) Pers]) (n = 39) were collected during four consecutive years (2006, 2007, 2008, and 2009) for complete chemical analysis. Forage samples were randomly collected every month from three pastures in which Santa Gertrudis (n = 144) cows were grazing at the King Ranch, Kingsville, TX (Latitude 27^{o} 31' N and Longitude 97^{o} 55' W). The average size of the pastures was 557 acres. The soil type of this area varies from clay to sandy loams. The vegetation type is predominated by grassland or savannah, with other species such as mesquite, cacti, and acacias (Gould, 1975).

Forage Collection

Warm-season perennial forage samples were hand-plucked at different locations of the pastures that animals were grazing, and were estimated to be similar to the forage that cows were consuming. Forage samples were stored in paper bags and transported to a laboratory at Texas A&M University, Kingsville, TX. Immediately after collection, forage

samples were freeze-dried, and the freeze-dried samples were sent to the ruminant nutrition laboratory at Texas A&M University, College Station, TX. Upon arrival at the ruminant nutrition laboratory at Texas A&M University, forage samples were dried at 65°C in an oven (Lindberg/Bluem Model: GO1305A1) until maintaining a constant weight (about 48 h) and then ground to pass a 2-mm screen using a ball mill (Thomos Scientific Model: 3375 - E25). The ground samples were stored in 120-ml snap-seal containers for subsequent physical analyses.

Chemical Analyses

All forage samples (2-mm ground) were sent to Cumberland Valley Analytical Services (Hagerstown, MD 21742) for the following chemical analyses: DM was performed in two steps; the first step was according to Goering and Van Soest (1970), and during the second step oven temperature increased to 105 ºC, according to National Forage Testing Association (2002); ash was determined according to AOAC (2002, method 942.05); CP and non-sequential ADF analyses were performed according to AOAC (2002; methods 2001.11 and 973.18), respectively; NDF analysis was determined according to Van Soest et al. (1991); ether extract (**EE**) was determined by AOAC (2002; method 920.39); and lignin analysis was performed according to Goering and Van Soest (1970) using 72% sulfuric acid, with modifications (Cumberland Valley Analytical Services, Inc., http://www.foragelab.com).

In Vitro Gas Production Measurement

The in vitro anaerobic fermentation and gas production was performed in fermentation chambers as described by Tedeschi et al. (2009). Briefly, the fermentation chamber has 22 sensors; divided into two sets, 1-11 and 12-22. In each set a blank bottle (only media + rumen fluid) and a bottle with alfalfa (*Medicago sativa* L.) hay was included as laboratory controls to set fermentation standards. Therefore, 22 bottles (which two were blanks, two had alfalfa hay, and 18 were those used to incubate the forage samples) were used in each run. The blanks were used to correct for atmospheric pressure variation and the gas produced by the fermentation of substrates in the rumen fluid and the media. Tedeschi et al. (2008a) reported that the adjustment for gas production had an impact on the fermentation curve. Alfalfa hay was used as a laboratory standard to compare the pattern of the fermentation across runs. Feed samples (200 mg of 2-mm ground samples) were transferred to a Wheaton bottle (125 ml), which contained a small Teflon-covered stir-bar inside to simulate ruminal movements, wetted with 2 ml of boiled distilled water to avoid sample dispersion, and media was added under anaerobic condition.

The in vitro medium used was the phosphate-bicarbonate medium and reducing solution of Goering and Van Soest (1970). Media and bottles were continuously ventilated with CO_2 to avoid contamination with O_2, and the pH was set to be between 6.8 and 6.9. Saturation was controlled by the color change of resazurin indicator from purple (rich in O_2) to pink/colorless (lack of O_2). Bottles were filled with 14 ml of media, closed with butyl rubber stoppers lightly greased, and crimp-sealed with aluminum caps. Strict anaerobic technique was employed in all transfers (Bryant, 1972; Hungate, 1950). The ruminal fluid

inoculum was obtained from a nonlactating, rumen-cannulated cow that had free access to medium quality mixed forages (mostly warm-season grasses). The ruminal fluid was filtered through four layers of cheesecloth and then through glass wool. The ruminal fluid was mixed continuously with CO_2 to minimize changes in microbial populations and to avoid O_2 contamination. At collection, the pH of the ruminal fluid was measured using a portable pH meter.

A needle was introduced into the rubber stopper to capture the gas pressure inside the bottles. A pressure sensor was attached to the needle and the pressure was recorded to a software (PicoLog, PicoTech, UK) as described by Tedeschi et al. (2008b). When the fermentation chamber temperature reached 39 ^{o}C, 4 ml of the ruminal fluid was added into each bottle. After adding ruminal fluid, the fermentation chamber was closed. When the temperature inside the fermentation chamber reached 39 ^{o}C, bottles were ventilated with a needle for 5 s to allow each bottle to start with the same pressure. The fermentation chamber was closed and when the fermentation reached 39 ^{o}C, data recording was initiated. Temperature inside the chamber was maintained at 39 ^{o}C during the fermentation period (48 h). Gas pressure was automatically recorded every 5 min using a computerized system similar to that described by Pell and Schofield (1993).

After 48 h of fermentation (2880 data points were taken by the computerized system), the anaerobic fermentation was stopped, bottles were depressurized, and pH measured using a digital pH meter. In order to determine the digestible NDF (**dNDF**), neutral detergent solution (40 ml) (Van Soest et al., 1991) was added to each bottle. Bottles were crimp-sealed, and cooked in an autoclave for 60 min at 105 ^{o}C, filtered by a

gravimetric method using a Whatman 54 filter paper using a vacuum system, and dried in oven for 72 h at 60 ^{o}C. After this period filters were weighed to estimate undegraded NDF, and forage digestibility was computed by difference.

Statistical Analysis

The pressure data measured in each bottle was converted to volume by using individual adjustments for each set of bottles and sensors, and standardized to 100 mg of sample. The volume of each forage sample was adjusted for the pressure of the blank bottles (average of two bottles). The adjusted volume data were fitted to nonlinear models using the Gas Production Fitting System v. 3.2 (**GasFit**, http://nutritionmodels.tamu.edu) (Tedeschi et al., 2008b) to obtain the kinetic parameters. The following parameters were analyzed: the asymptote (maximum gas production), ml; the fractional rate of gas production, h^{-1}; and the lag time, h. Preliminary analysis indicated the two-pool logistic model (Eq. [1]) had the best fit.

$$\text{Gas volume} = a/(1+\exp(2+4\times b\times(c-t)))+d/(1+\exp(2+4\times e\times(c-t))) \quad \text{Eq.[1]}$$

where a and d are the asymptote of the fast and slow substrate pools, ml; b and e are the fractional degradation rates of the fast and slow substrate pools, h^{-1}; c is the lag time, h; and t is time, h.

Comparison of equations was performed using the Model Evaluation System v. 3.1.4 (**MES**; http://nutritionmodels.tamu.edu) as described by Tedeschi (2006). Briefly, the mean square error of prediction (**MSEP**), the concordance correlation coefficient (**CCC**), and linear regression analysis were used.

Calculation of Total Digestible Nutrients

The TDN is a method to measure feed energy value. According to Weiss (1992), standard analytical analysis alone cannot be used to determine feed energy value. Moran (2005) reported three methods to predict feed digestibility, TDN, and ME. Digestibility is not a direct way to measure energy but it is related to feed quality. The ME is measured as calories or joules per kilogram of DM, and TDN is the sum of the percentages of CP, crude fiber (**CF**), EE, and nitrogen free extract (**NFE**) that are digested in the gastrointestinal tract of the animal (Weiss, 1992).

Weiss (1992) proposed a theoretical equation to calculate TDN using concentrations of NDF, lignin, CP, ash, fatty acids or EE, and acid and neutral detergent insoluble crude protein (**ADICP** and **NDICP**, respectively). The equation has digestion coefficients for CP, lipids, and non-fiber carbohydrate; and it computes digestibility of NDF based on the ratio of lignin to NDF. The metabolic fecal TDN is subtracted to compute apparent TDN. The original equation proposed by Weiss et al. (1992) was modified to be used within the Cornell Net Carbohydrate and Protein System (**CNCPS**; Fox et al., 2004) as the level 1 solution for energy supply. Equation [2] has the form used by the level 1 solution of the CNCPS model.

$$\text{Apparent TDN} = 0.98\times(100 - NDF_N - CP - EE - ASH) + k_{dCP}\times CP + 2.25\times(EE - 1) + 0.75$$
$$(NDF_N - LIG)\times[1 - (LIG/NDF_N)^{0.667}] - 7 \qquad \text{Eq. [2]}$$

where NDF_N is the NDF adjusted for nitrogen (NDF – NDF insoluble N), % DM; EE is ether extract, % DM; k_{dCP} is the CP digestibility, %; and LIG is lignin, % DM.

One weakness of Eq. [2] is that it does not allow for changes in the digestibility of the NDF among feedstuffs. The main reason is that the values computed by Eq. [2] are the TDN for animals with DMI at maintenance level (**TDN_{1x}**); that means, values are not discounted for the level of intake as discussed by Tedeschi et al. (2005). In order to allow for changes in the digestibility of NDF, Tedeschi et al. (2009) developed an equation that computes the digestibility of the NDF by using the fractional rates of degradation (**kd**) of fiber and passage (**kp**), assuming a linear relationship in the dynamics of fermentation and passage in the rumen. Therefore, different kp were tested (4, 6 and 8%/h) and compared with values predicted by Eq. [3]. In this model, a 20% intestinal digestibility of NDF (**IDNDF**) was assumed as proposed by Sniffen et al. (1992) for available NDF for all forages. The IDNDF is an adjustment for fiber fermentation in the hindgut.

$$\text{Apparent TDN} = 0.98\times(100 - NDF_N - CP - EE - ASH) + k_{dCP}\times CP + 2.25\times(EE - 1) + (NDF - NDIN)\times(kd/(kd + kp) + IDNDF) - 7 \quad \text{Eq. [3]}$$

where NDF_N is the NDF adjusted for nitrogen (NDF – NDF insoluble N), % DM; EE is ether extract, % DM; k_{dCP} is the CP digestibility, %; LIG is lignin, % DM; kd is fractional rate of NDF degradation, h^{-1}; kp is fractional rate of passage, h^{-1}; and IDNDF is the intestinal digestibility of NDF, % DM.

An adjustment for unavailable carbohydrate (**CHOC**) as proposed by Sniffen et al. (1992) and evaluated by Traxler et al. (1998) was also investigated as shown in Eq. [4].

Apparent TDN = $0.98\times(100 - NDF_N - CP - EE - ASH) + k_{dCP}\times CP + 2.25\times(EE - 1) + (NDF - NDIN - 2.4\times Lignin)\times(kd/(kd + kp) + IDNDF) - 7$ Eq. [4]

where NDF_N is the NDF adjusted for nitrogen (NDF – NDF insoluble N), % DM; EE is ether extract, % DM; k_{dCP} is the CP digestibility, %; LIG is lignin, % DM; kd is fractional rate of NDF degradation, h^{-1}; kp is fractional rate of passage, h^{-1}; Lignin is % DM; and IDNDF is the intestinal digestibility of NDF, % DM.

Simulations of Energy Balance of Grazing Cows

Simulations to predict animal requirements of ME (Mcal/d) and MP (g/d) for maintenance, pregnancy, lactation, and growth and supply of ME and MP by the pastures were performed using the Large Ruminant Nutrition System v. 1.0.1 (**LRNS**; http://nutritionmodels.tamu.edu), which is based on the CNCPS v. 5 as published by Fox et al. (2004). All simulations were performed with level 2 of solution of the LRNS model. The level 2 of solution used the mechanistic ruminal sub-model (Fox et al., 2004). For each month of the four years of forage sampling, simulations were performed using actual data, including the chemical analyses of the forages and supplement (Table 3.1), and average temperature, average humidity, wind speed (Table 3.2), and animal information (except for DMI which was not measured). Cows consumed (1.45 kg/d) a protein supplement (29% CP). However, during the months of July and August for all periods cows did not receive any supplementation.

The animal information included days pregnant, days since calving, BW, expected calf birth weight, BCS, actual supplement intake, and predicted forage intake by the LRNS

model. The cow average BW for period 1 (May 2006 – April 2007; **P1**) was 553 ± 71 kg, for period 2 (May 2007 – April 2008; **P2**) was 572 ± 43kg, and period 3 (May 2008 – April 2009; **P3**) was 580 ± 51kg (Figure 3.1). All animals were weighed three times (January, July and September) during each period as per the management procedures of the King Ranch. Thus, in order to estimate cow monthly BW, linear interpolation equation was used.

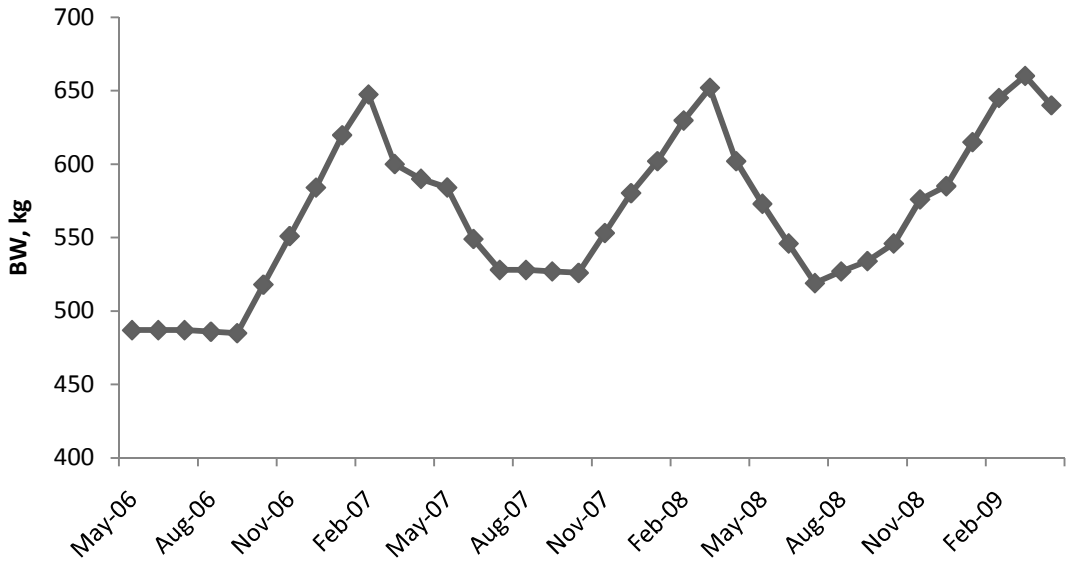

Figure 3.1: Cow BW variation during the collection period. Cows calved in March and calves were weaned in October

Table 3.1: Chemical analyses of collected forages in South Texas

Month	DM, %	ADF	NDF	Lignin	EE	Ash	CP	ADIN	NDIN	SP[1]
2006					%, DM					
Feb	92.6	42.3	85.1	6.0	1.3	9.1	5.2	2.2	3.0	-
Mar	91.7	51.5	76.5	9.5	1.1	9.1	5.2	1.4	2.1	32.4
Apr	93.1	34.5	80.2	8.8	1.4	9.1	7.5	3.4	4.3	-
May	88.8	32.8	70.4	7.4	1.6	10.0	12.1	4.0	16.8	-
Jun [2]	95.2	56.8	80.7	10.2	1.1	7.9	2.6	1.2	1.3	28.5
Jul	90.8	38.0	65.5	8.3	1.7	9.9	12.0	3.3	16.1	-
Aug	90.7	38.0	62.1	8.3	1.4	9.2	11.9	4.2	7.0	-
Sep	92.4	39.9	64.0	6.5	1.2	8.8	12.0	3.9	10.5	-
Oct	94.9	41.5	75.3	5.9	1.3	9.7	8.8	1.3	2.8	37.2
Nov	93.1	44.2	73.6	8.5	0.9	8.5	7.4	3.6	7.8	-
Dec	95.7	45.5	76.4	8.0	1.0	9.3	7.2	1.5	2.6	37.9
2007										
Jan	94.7	48.1	80.3	10.2	1.2	6.0	7.3	2.2	2.8	31.1
Feb	93.1	51.1	77.4	11.6	1.2	10.2	6.9	4.8	9.7	-
Mar	93.1	53.8	74.0	10.5	1.8	2.0	6.8	1.5	2.5	27.3
Apr	93.2	47.9	72.2	9.3	1.7	3.4	6.9	1.3	2.2	37.0
May	93.1	37.0	69.0	5.7	1.7	5.1	11.7	1.3	3.3	45.7
Jun	91.7	46.2	72.6	8.8	1.3	4.3	6.2	1.3	2.3	36.7
Sep	93.8	39.2	67.6	7.3	1.9	12.6	11.2	1.5	3.5	43.6
Oct	92.5	40.5	71.1	7.7	1.6	9.8	11.9	1.8	3.9	38.7
Nov	93.4	47.6	75.4	8.0	1.2	9.1	4.9	1.5	3.9	35.3
Dec	94.6	44.6	74.4	9.1	1.2	8.1	4.7	1.5	3.6	26.6
2008										
Jan	94.4	50.2	76.3	8.7	1.1	10.6	5.3	1.8	2.0	22.3
Feb	91.7	51.7	76.3	10.1	1.1	10.6	6.2	1.9	2.2	28.2
Mar	91.0	46.3	71.9	10.1	1.2	11.3	10.0	2.6	4.3	22.0
Apr	92.4	57.1	80.3	11.5	0.4	9.7	4.4	1.8	2.0	23.4
May	92.0	60.8	80.3	11.3	0.7	12.1	4.2	1.6	1.9	27.2
Jun	92.6	47.5	76.1	10.2	1.1	8.9	8.7	2.6	3.3	30.9
Jul	93.3	36.2	71.8	6.0	2.2	10.6	11.2	1.6	3.1	38.2
Aug	93.5	35.9	68.1	6.3	3.1	10.4	12.9	1.6	4.7	38.8
Sep	92.7	38.1	70.5	6.4	2.2	11.0	10.4	1.4	4.0	32.0
Oct	93.3	45.2	74.0	7.9	1.1	8.9	7.3	1.9	4.2	36.1
Nov	93.5	44.1	75.7	7.2	1.2	11.3	7.7	1.9	3.9	30.2
2009										
Jan	93.1	47.4	77.8	8.3	1.1	10.8	7.2	1.7	2.8	35.7
Feb	91.6	48.3	78.5	9.7	1.0	9.4	8.4	2.0	2.9	34.9
Mar	87.1	50.6	82.0	9.9	1.0	6.2	6.5	2.1	2.2	42.0
Apr	93.0	51.1	81.2	11.1	0.7	6.5	6.5	2.2	2.4	33.9
May	91.2	54.3	81.3	11.0	0.9	7.9	5.8	2.0	2.1	33.0
Jun	86.5	41.4	68.9	6.2	2.1	11.4	9.3	1.6	2.9	34.4
Aug	91.9	49.5	79.1	10.6	0.9	7.4	7.3	2.2	3.0	34.5
Supplement	89.0	15.2	31.9	3.5	3.9	7.6	29.0	1.3	5.0	21.0

[1] Soluble Protein, % of CP

[2] The CP value is less than expected.

Table 3.2: Climatic data at the King Ranch, Kingsville, TX for the period of forage collection

	Jan	**Feb**	**Mar**	**Apr**	**May**	**Jun**	**Jul**	**Aug**	**Sep**	**Oct**	**Nov**	**Dec**	**AVG**
							2006						
Temp ºC	16.91	18.78	22.47	26.05	26.71	28.53	29.27	30.24	26.78	23.53	18.59	15.14	23.58
Dew Point (ºC)	8.07	7.56	13.83	19.23	19.21	21.62	23.24	22.65	21.13	17.69	11.30	9.98	16.29
Humidity (%)	59.52	55.52	60.85	67.12	64.17	64.52	67.88	63.77	72.25	70.96	70.37	76.79	66.14
Wind (km/h)	14.48	15.64	17.58	16.16	15.83	11.72	13.90	12.13	11.33	13.84	11.86	11.55	13.84
Prec. (mm)		2.80	7.50	0.00	72.10	152.30	101.40	20.10	197.50	35.50	0.00	64.90	59.46
							2007						
Temp ºC	11.77	15.40	20.56	21.09	25.26	27.61	27.85	28.37	27.32	23.49	18.91	16.90	22.04
Dew Point (ºC)	7.97	9.44	14.55	15.70	20.16	23.13	23.85	23.82	22.62	16.16	12.98	9.73	16.68
Humidity (%)	81.55	74.50	74.35	78.10	76.64	78.53	83.19	79.42	79.48	71.87	75.90	68.23	76.81
Wind (km/h)	14.80	12.13	13.91	11.80	9.81	11.64	11.76	7.21	5.27	7.01	9.01	13.60	10.66
Prec. (mm)	103.00	0.00	49.10	62.00	228.90	71.10	356.87	57.91	110.40	12.00	7.20	0.00	88.21
							2008						
Temp ºC	13.53	18.87	19.73	23.15	27.78	29.72	27.97	28.46	26.28	22.54	18.85	15.65	22.71
Dew Point (ºC)	7.04	11.23	11.31	15.07	20.66	22.80	22.78	23.71	20.04	15.34	11.83	7.85	15.80
Humidity (%)	72.55	70.76	66.48	67.23	70.81	67.03	76.77	78.19	73.47	71.26	73.03	67.55	71.26
Wind (km/h)	14.95	12.93	16.92	15.24	16.72	16.95	11.99	9.60	7.46	7.27	8.80	15.21	12.84
Prec. (mm)	35.80	0.00	0.80	34.80	20.60	35.70	182.60	172.00	102.40	24.90	0.00	5.84	51.29
							2009						
Temp ºC	15.38	18.59	19.30	23.70	27.28	29.41	31.02	30.22					24.36
Dew Point (ºC)	5.99	10.75	12.01	13.91	20.30	22.52	22.87	22.54					16.36
Humidity (%)	63.03	68.68	71.81	63.77	70.45	69.90	67.29	67.42					67.79
Wind (km/h)	13.15	14.14	15.57	16.36	13.34	14.05	15.00	11.63					14.16
Prec. (mm)	0.00	4.10	8.70	9.40	65.30	19.50	0.00	38.90					18.24

Results and Discussion

In Vitro Gas Production

The two-pool logistic model had the best fit for all forage samples. Similar to these findings, Schofield et al. (1994) concluded that single-pool models over-predict values for single substrates when different substrate pools were digested separately and the parameters were deficient in biological meaning. They also concluded that the variation in mixed substrates cannot be replicated by the exponential curve with dual pool. Van Soest (1994) showed that lignin level was not considered in multiple pools. Doane et al. (1997) concluded the best model to fit bromegrass (*Bromus inermis L.*) was the one-pool logistic model although the NDF (55.6%) value of these cool season grass was lower than the warm-season forage values (73.9%) from South Texas. The results of IVGP are presented in Table 3.3.

There was a lag time in all fermentations. Similar results were found by Schofield and Pell (1993) working with a cool-season perennial grass; timothy [*Phleum pretense L.*], and a warm-season perennial guineagrass [*Panicum maximum Jacq.*]). The average lag times in this South Texas experiment were 6.47 ± 0.54; 7.75 ± 0.65; 7.49 ± 2.01; and 5.44 ± 1.46 h for 2006, 2007, 2008, and 2009, respectively. Schofield and Pell (1995) reported a lag time for timothy and guineagrass of 6.58, and 6.93 h, respectively. Miller and Hobbs (1994) suggested the delay in the fermentation might be because IVGP uses dry forage and the forage was not accessible to microbes until they became hydrated. Their results were similar to Schofield and Pell (1995) in which they did not hydrate the samples.

Pell and Schofield (1993) working with alfalfa, bromegrass, timothy, and stargrass (*Cynodon nlemfuensis Harl.*) reported a relationship of 0.37 ml of gas produced by 1 mg of

DM. Similar results were found by Schofield and Pell (1995) working with timothy, alfalfa, red clover (*Trifolium pretense L.)*, and guineagrass (0.39 ml/mg). Ratios of 0.41 ± 0.11 ml/mg in 2006, 0.34 ± 0.09 ml/mg in 2007, 0.34 ± 0.07 in 2008, and 0.26 ±0.10 in 2009 were found in the South Texas warm-season grasses. This difference can be explained by the different digestibility levels in the warm-season grasses 36.8 ± 4.2% in 2006, 37.7 ± 4.1 % in 2007, 34.5 ± 5.6 % in 2008, and 33.84 ± 4.02 % in 2009 compared to ones reported by Schofield and Pell (1995) for timothy of 61.8 % and guineagrass of 58%.

The volumes of total gas produced by the second pool were 10.95 ± 2.00 ml, 9.10 ± 1.92 ml, 8.60 ± 1.91 ml, and 6.51 ± 1.90 ml; respectively for 2006, 2007, 2008, and 2009. Schofield and Pell (1995) reported total gas production of 15.9 and 16.19 ml for timothy and guineagrass, respectively. The digestibility and the quality of the warm-season grasses from this South Texas experiment were less compared to those grasses. The fractional degradation rates in these forages were 0.034 ± 0.005 h^{-1} in 2006, 0.033 ± 0.005 h^{-1} in 2007, 0.028 ± 0.004 h^{-1} in 2008, and 0.029 ± 0.005 h^{-1} in 2009. Although the digestibilities of their forages were superior, similar results were found by Schofield and Pell (1995) for timothy 0.032 h^{-1} and 0.033 h^{-1} for guineagrass.

Total Digestible Nutrients

Allen and Mertens (1988) suggested that inside the rumen there is a selection of particle size for passage (and digestion) and it cannot be measured on in vitro studies. Further discussion of the mathematics of fractional passage and digestion rates were provided by Vieira et al. (2008a,b). In their work, the fractional passage rate can be modeled

Table 3.3: In vitro gas fermentation parameters of the isolated NDF

Parameters [1]	Jan	Feb	Mar	April	May	Jun	July	Aug	Sep	Oct	Nov	Dec
						--- 2006 ---						
a, ml	-	5.57	5.29	5.69	5.46	4.27	6.54	6.23	7.37	6.57	7.25	5.84
b, h^{-1}	-	0.164	0.140	0.117	0.122	0.136	0.174	0.149	0.135	0.150	0.155	0.118
c, h	-	6.87	6.94	6.48	6.11	7.26	6.69	5.71	6.62	6.87	6.05	5.54
d, ml	-	8.41	10.58	12.14	9.86	8.84	10.65	11.86	14.96	13.26	11.09	8.88
e, h^{-1}	-	0.045	0.032	0.030	0.029	0.036	0.039	0.031	0.032	0.032	0.039	0.033
						--- 2007 ---						
a, ml	3.82	4.25	4.59	4.10	5.32	-	-	4.77	5.19	5.62	4.34	5.65
b, h^{-1}	0.161	0.171	0.179	0.181	0.170	-	-	0.176	0.131	0.099	0.117	0.086
c, h	8.35	8.38	7.83	7.99	7.25	-	-	8.00	8.26	7.13	7.99	6.33
d, ml	7.03	9.41	10.18	10.60	11.22	-	-	11.53	9.75	8.51	6.46	6.34
e, h^{-1}	0.040	0.036	0.037	0.036	0.040	-	-	0.034	0.030	0.025	0.032	0.023
						--- 2008 ---						
a, ml	4.89	4.54	4.89	3.70	3.08	4.63	4.59	6.77	4.81	3.18	4.33	-
b, h^{-1}	0.10	0.11	0.10	0.11	0.11	0.14	0.14	0.13	0.13	0.10	0.12	-
c, h	6.08	5.85	5.66	6.59	9.03	8.03	10.87	8.85	10.27	4.94	6.21	-
d, ml	9.15	6.55	7.54	7.12	6.32	10.65	9.35	10.67	10.26	6.07	10.96	-
e, h^{-1}	0.026	0.022	0.030	0.027	0.023	0.032	0.031	0.032	0.030	0.026	0.026	-
						--- 2009 ---						
a, ml	6.109	3.87	4.75	5.53	4.75	6.90	-	4.14	-	-	-	-
b, h^{-1}	0.08	0.131	0.087	0.049	0.094	0.132	-	0.109	-	-	-	-
c, h	3.73	7.69	4.96	3.58	5.83	6.22	-	6.11	-	-	-	-
d, ml	7.047	7.29	6.06	6.18	4.77	10.01	-	4.23	-	-	-	-
e, h^{-1}	0.028	0.029	0.021	0.032	0.024	0.036	-	0.031	-	-	-	-

[1] a = total gas production, ml 100 mg of DM (1^{st} pool), b = kd, %/hr (1^{st} pool), c = Lag time, h, d = total gas production, ml 100 mg of DM (2^{nd} pool), e = kd, %/hr (2^{nd} pool)

using a gamma distribution for the intrinsic transformations that a particle has to undergo in the rumen before it can escape. Furthermore, during filtration most of the microorganisms that degrade fiber stay attached to the solid part of the rumen material (Meyer and Mackie, 1986) and they will appear in the undigested portion of the NDF.

Despite these restrictions, the in vitro DM digestibility estimate of the IVGP technique is highly correlated with that predicted by in vivo methods (Marten and Barnes, 1980). Van Soest (1991), however, reported that even though systems of chemical analyses are fast and accurate, they do not reflect the biological and nutritional reality that can be reached with in vitro systems.

The TDN values calculated by Eqs. [2] and [3], and by the LRNS are presented in Tables 3.4 and 3.5. Table 3.6 has the adequacy statistics. The average values for TDN_{1x} without adjustment for CHOC (Sniffen et al., 1992) were 55.9 ± 5.09%, 49.35 ± 5.28%, and 45.0 ± 5.27 %, respectively, assuming fractional passage rates of 4, 6, and 8 %/h (Table 3.6). The average TDN_{1x} predicted by Weiss et al. (1992) was 53.8 ± 3.45 (Table 3.6). When compared to the predictions by Weiss et al. (1992), the analysis of model adequacy indicated that TDN_{1x} predicted by Eq. [3] assuming a kp of 4 %/h had a high accuracy (**Cb**) of 0.82 and mean bias of -2.19 % TDN_{1x}, even though the precision was the least ($r^2 = 0.59$), points were scattered. The prediction using a kp of 8% had the greatest precision ($r^2 = 0.67$) but the least accuracy (Cb = 0.31). When the adjustment for CHOC was performed (Eq. [4]), the TDN_{1x} values decreased considerably to 42.8 ± 5.68%, 38.1 ± 5.73%, and 35.1 ± 5.74% for kp of 4, 6, and 8 %/h; respectively (Table 3.6). This suggested the inclusion of CHOC in

predicting TDN_{1x} was likely to significantly decrease the predicted performance of the animals.

The average TDN values calculated by the LRNS model using the predicted DMI were 48.8 ± 4.54% and 45.0 ± 5.47 %, respectively, for levels of solution 1 (Eq. [2]) and 2. The level of solution 2 uses the mechanistic rumen submodel and the individual fractional degradation rates of the feed carbohydrate and protein fractions (Fox et al., 2004). Both TDN values predicted by levels 1 and 2 were discounted by the level of intake as discussed by Tedeschi et al. (2005). When DMI was used as 2.6% of BW, the TDN values for levels 1 and 2 were 47.8 ± 4.46 and 42.7, respectively. The decrease in TDN values was expected since the LRNS accounts for level of intake to predict fractional passage rate; as DMI increases the fractional passage rates also increases, and therefore, the predicted TDN decreases (Fox et al., 2004).

The average DMI reported in the literature (Hatfield et al., 1989; Juarez Lagunes et al., 1999; and Sowell et al., 2003) for free ranging beef cows was 2.6% of BW, suggesting the LRNS may have underestimated the DMI intake for grazing cows in South Texas. Therefore, the values obtained by the LRNS (either using predicted DMI or the 2.6% BW to predict DMI) were likely to yield more realistic numbers because the model simultaneously accounts for CHOC and discounts for level of intake. The values predicted by Eq. [2], [3], and [4] were the TDN_{1x}; thus, they have to be discounted as suggested by Tedeschi et al. (2005) for more realistic predictions of animal performance when using a model that does not account for these factors in predicting nutritive values of feeds. When TDN was compared and predicted by the level 2 of solution of the LRNS

Table 3.4: Predicted TDN for 2006 and 2007 using two theoretical equations and the Large Ruminant Nutrition System using predicted DMI or assuming DMI as 2.6% of BW for two levels of statistical solutions

Items	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
						--- 2006 ---						
Weiss et al. (1992)		53.3	51.4	50.5	56.2	50.9	56.9	55.2	57.9	55.8	55.3	55.1
TDN1x, no adjustment for CHO C												
kp = 4 h^{-1}		58.9	55.1	52.1	57.8	57.0	63.9	58.6	60.2	55.6	61.6	57.6
kp = 6 h^{-1}		50.6	47.9	44.8	52.8	49.2	59.0	53.4	55.0	48.5	55.1	50.4
kp = 8 h^{-1}		45.0	43.2	40.2	49.6	44.0	55.7	50.0	51.6	44.0	50.7	45.8
TDN1x, adjusted for CHO C												
kp = 4 h^{-1}		48.4	40.3	38.8	46.9	40.5	50.1	46.0	50.1	46.4	47.5	45.1
kp = 6 h^{-1}		41.6	35.3	33.5	43.5	35.1	47.2	42.6	46.4	40.7	43.0	39.8
kp = 8 h^{-1}		37.0	32.1	30.2	41.4	31.6	45.2	40.5	44.0	37.1	40.0	36.3
LRNS, using predicted DMI												
Level 1		47.0	44.0	47.0	52.0	47.0	54.0	55.0	56.0	52.0	50.0	49.0
Level 2		42.0	39.0	42.0	47.0	39.0	49.0	47.0	53.0	52.0	42.0	46.0
LRNS, using DMI = 2.6% BW												
Level 1		47.0	44.0	46.0	51.0	45.0	53.0	54.0	54.0	51.0	49.0	48.0
Level 2		44.0	37.0	38.0	44.0	34.0	47.0	46.0	51.0	51.0	41.0	45.0
						--- 2007 ---						
Weiss et al. (1992)	52.3	49.6	58.9	59.1	62.7	58.2			53.8	54.9	53.9	53.9
TDN1x, no adjustment for CHO C												
kp = 4 h^{-1}	60.8	58.0	66.2	64.7	66.2	62.3			54.7	52.8	55.9	51.6
kp = 6 h^{-1}	53.0	51.3	59.2	57.8	59.6	55.4			48.6	46.7	49.0	45.3
kp = 8 h^{-1}												
TDN1x, adjusted for CHO C	47.9	47.0	54.5	53.3	55.2	50.9			44.8	43.0	44.5	41.5
kp = 4 h^{-1}	43.6	39.3	49.2	49.8	56.6	48.4			43.7	42.1	43.5	39.3
kp = 6 h^{-1}	38.3	35.4	44.6	45.1	51.4	43.6			39.3	37.7	38.5	35.0
kp = 8 h^{-1}												
LRNS, using predicted DMI	34.8	32.8	41.6	42.0	48.0	40.5			36.5	35.0	35.2	32.4
Level 1	48.0	45.0	55.0	56.0	59.0	54.0			52.0	52.0	49.0	49.0
Level 2												
LRNS, using DMI = 2.6% BW	45.0	39.0	49.0	51.0	58.0	53.0			53.0	51.0	46.0	46.0
Level 1	47.0	43.0	54.0	54.0	58.0	53.0			50.0	51.0	49.0	48.0
Level 2	43.0	37.0	48.0	49.0	56.0	50.0			50.0	50.0	44.0	43.0

Table 3.5: Predicted TDN for 2008 and 2009 using two theoretical equations and the Large Ruminant Nutrition System using predicted DMI or assuming DMI as 2.6% of BW for two levels of statistical solutions

Items	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
						--- 2008 ---						
Weiss et al. (1992)	50.6	48.9	49.3	48.7	47.2	50.1	56.5	58.8	56.4	54.1	52.2	
TDN1x, no adjustment for CHO C												
kp = 4 h^{-1}	49.6	46.7	52.9	51.1	46.0	54.5	56.1	59.6	56.1	52.7	49.7	
kp = 6 h^{-1}	42.8	40.2	46.5	43.8	39.1	47.5	49.5	53.5	49.8	46.3	43.1	
kp = 8 h^{-1}	38.5	36.3	42.4	39.2	34.9	43.0	45.3	49.6	45.7	42.3	39.0	
TDN1x, adjusted for CHO C												
kp = 4 h^{-1}	37.1	33.2	37.8	34.3	30.8	38.7	46.9	49.9	46.5	41.4	39.4	
kp = 6 h^{-1}	32.2	28.8	33.7	29.6	26.3	34.1	41.7	45.2	41.6	36.8	34.4	
kp = 8 h^{-1}	29.2	26.2	31.0	26.7	23.5	31.1	38.4	42.2	38.5	33.9	31.3	
LRNS, using predicted DMI												
Level 1	47.0	46.0	47.0	42.0	41.0	44.0	54.0	53.0	50.0	48.0	46.0	
Level 2	46.0	45.0	41.0	40.0	37.0	40.0	52.0	52.0	45.0	42.0	40.0	
LRNS, using DMI = 2.6% BW												
Level 1	46.0	45.0	46.0	41.0	40.0	43.0	53.0	52.0	49.0	47.0	45.0	
Level 2	43.0	43.0	39.0	37.0	32.0	37.0	49.0	50.0	43.0	40.0	38.0	
						--- 2009 ---						
Weiss et al. (1992)	50.7	51.5	53.9	52.1	51.2	56.0		52.7				
TDN1x, no adjustment for CHO C												
kp = 4 h^{-1}	50.1	53.6	49.4	57.2	50.5	58.3		56.4				
kp = 6 h^{-1}	43.1	46.4	42.6	49.6	43.4	51.8		49.1				
kp = 8 h^{-1}	38.7	41.9	38.5	44.7	39.1	47.6		44.5				
TDN1x, adjusted for CHO C												
kp = 4 h^{-1}	38.0	39.1	36.5	40.0	35.3	48.3		40.3				
kp = 6 h^{-1}	32.9	34.1	31.7	34.9	30.6	43.3		35.5				
kp = 8 h^{-1}	29.6	31.0	28.8	31.7	27.7	40.0		32.4				
LRNS, using predicted DMI												
Level 1	44.0	43.0	45.0	43.0	44.0	51.0		44.0				
Level 2	44.0	42.0	43.0	41.0	35.0	46.0		36.0				
LRNS, using DMI = 2.6% BW												
Level 1	43.0	42.0	44.0	42.0	43.0	50.0		43.0				
Level 2	42.0	39.0	40.0	37.0	30.0	43.0		34.0				

using 2.6% BW as the predicted DMI with the TDN accounted for CHOC (Eq. [5]), the mean bias was negligible (-0.126 %) and the accuracy was 0.999, assuming kp of 4% (Table 3.6). The comparison of LRNS predicted by level 2 with the LRNS predicted DMI had less precision and accuracy (Table 3.6). This suggests that discounting the TDN for CHOC (Eq. [5]) was likely to yield more realistic values when compared to the values predicted by the LRNS model.

Pacheco et al. (1982) working with Kleberg bluestem hay in South Texas reported a mean TDN value of 49.7 ± 5.54% with a range varying from 45.1 to 54.4%. Nelsen et al. (1982) reported a 61% TDN value for Coastal bermudagrass [*Cynodon dactylon* (L.) Pers.]. Hawkings et al. (1964) reported a mean value of 54.9% with a range from 52.3 to 62.2% for Coastal bermudagrass hay. Those differences may be explained by differences in the environment, season, stage of maturity and soil. Menke and Steigass (1998) reported several regression equations to predict energy values in roughage using IVGP in 24 h fermentation. The authors also suggested that comparing regression equations between roughage, silages, mixed feeds, dairy rations, and all feedstuffs together, the best results were found in roughage because the range in chemical composition and energy values were larger, although the mean variation was greater. The authors concluded that different equations have to be used for different feedstuffs. As expected, as kp increased from 4 to 8 %/h, the TDN values decreased. There were high Pearson correlations between TDN_{1x} computed at 4, 6, and 8 %/h of kp (**TDN_4**, **TDN_6**, and **TDN_8**, respectively) with the kd of the first pool (0.68, 0.62, and 0.57, respectively) and the second pool (0.84, 0.74, 0.67, respectively, Table 3.7). The high correlation with the second pool was expected because the kd of the second

Table 3.6: Model adequacy statistics of the comparison between different methods in predicting TDN [1]

Comparisons	**Mean**	**SD**	**Median**	**r^2**	**MSEP**	**MB**	**Cb**	**CCC**	**AIC**
Weiss et al. (1992), Eq. [2] with:	53.8	3.45	53.9						
Eq. [3] with kp of 4%/h	55.9	5.09	56.1	0.59	15.3	-2.19	0.82	0.63	64.8
Eq. [3] with kp of 6%/h	49.3	5.22	49.1	0.64	30.4	4.51	0.61	0.49	59.6
Eq. [3] with kp of 8%/h	45.0	5.27	44.5	0.67	87.0	8.79	0.31	0.25	56.6
LRNS Level 2 and predicted DMI with:	45.0	5.47	45.0						
Eq. [4] with kp of 4%/h	42.8	5.68	42.1	0.54	21.0	2.23	0.93	0.68	105.4
Eq. [4] with kp of 6%/h	38.1	5.73	37.7	0.55	64.0	6.96	0.56	0.42	104.3
Eq. [4] with kp of 8%/h	35.0	5.74	34.8	0.56	115.1	9.98	0.39	0.29	103.9
LRNS Level 2 and 2.6% BW as DMI with:	42.7	5.97	43.0						
Eq. [4] with kp of 4%/h	42.8	5.68	42.1	0.60	14.9	-0.13	1.00	0.78	106.7
Eq. [4] with kp of 6%/h	38.1	5.73	37.7	0.61	35.9	4.60	0.76	0.60	106.3
Eq. [4] with kp of 8%/h	35.0	5.74	34.8	0.60	73.0	7.62	0.54	0.42	106.6

[1] SD is standard deviation
MSEP is mean square error of prediction
MB is mean bias
Cb is accuracy
CCC is concordance correlation coefficient
AIC is the Akaike's Information Criteria
LRNS is the Large Ruminant Nutrition System model v. 1.0.1

pool (fiber) was used to compute TDN. The correlation between TDN_4, TDN_6, and TDN_8 with TDN_{Weiss} (Eq. [2]) were high (0.62, 0.66, and 0.68) and increased with kp values.

According to Getachew et al. (2005), IVGP data can be used to predict the energy value of forages. Menke et al. (1979) reported a high correlation (r = 0.98) for predicting ME values of feedstuffs using IVGP. Other values in the literature indicated a 15% variation in ME values predicted by IVGP techniques compared with those of other in vivo techniques (Krishnamoorthy et al, 1995). Those authors concluded that IVGP can be used to predict energy values. Concurring with those authors, Iantcheva et al. (1999) also reported that IVGP can be used to estimate energy values in forages, and used regression equations for alfalfa (r = 0.86 to 0.93), and grass hay (r = 0.83 to 0.91).

Based on evaluations of the South Texas warm-season forages, a kp of 4 %/h may reflect the typical passage rate in beef cows grazing low to moderate forage quality. The average TDN for kp of 4%/h was 55.9% (Table 3.6). The NRC (2000) suggested that TDN ranged from 53 to 57% in forages when the passage rate was 4%/h. The predicted kp by the LRNS model using the level 2 solution averaged 3.66 %/h. This value is in agreement with the assumption of using 4 %/h as the expected kp of these Santa Gertrudis cows. The comparison of TDN_4 and TDN_{Weiss} indicated the in vitro system may overpredict TDN_{Weiss} (55.9 vs 53.8; respectively, Table 3.6).

Relationships of Chemical Analyzes and Climatic Factors

Pearson correlations among chemical measurements, TDN, and climate variables are presented in Table 3.7. The weather in South Texas can be extremely variable, with

extended periods of drought, high summer temperatures, but generally mild winters. Class of the forage (grass vs legume, annual vs perennial, and cool-season vs warm season grass) light, temperature, and maturity are the most important factors affecting forage quality, followed by moisture (Van Soest, 1994). According to Volenec and Nelson (2003), climate and weather variations have great influence in the growth and establishment of plants in which light, temperature, and soil moisture are the main factors that influence vegetative development and reproduction. The allocation of photosynthetic resources into different plant tissues affects in nutritive value; thus, climate has an influence on their composition and nutritive value (Van Soest, 1994).

Moreira et al (2004) working with stargrass reported positive correlations between leaf NDF and leaf ADF with digestibility (r = 0.73 and 0.46, respectively); however, they found a negative correlation between NDF and ADF with digestibility (r = -0.58 and -0.56, respectively). The negative correlation between digestibility and fibrous parameters might be related to the ratio of stem to leaf, and it is likely that the forages in this study had a greater stem to leaf ratio. Nelson and Moser (1994) reported that forage quality decreases when the stem to leaf ratio increases. Yayneshet et al (2009), Moore and Jung (2001), and Casler and Jung (2006) reported a negative correlation between digestibility with lignin and NDF. Those data agree with that of the South Texas warm-season grasses in which there was a negative correlation (r = -0.43) between lignin and in vitro DM digestibility.

The positive correlation between CP and digestibility (r = 0.58) agrees with results reported by Getachew et al. (2004) and Ammar et al (2004). When compared to Archer and Decker (1977) and Ammar et al. (2004), the negative correlation between fibrous parameters

of Kleberg grass and Coastal bermudagrass; however, correlations were greater. In contrast to Getachew (2004), there was a positive correlation between CP and d (r = 0.49), a small correlation between e and CP (r = 0.13), and a positive correlation between SP and d.

The small negative correlation between lignin and temperature was not expected but is in agreement with Buxton and Redfearn (1997), who reported that lignified tissues provide resistance to support low temperatures and protection against diseases and insects. Ford et al. (1979) working with tropical and temperate grasses reported a positive correlation between temperature and lignin. The variance in those results may be explained by the difference in plant maturity. The weak correlation between lignin and parameters b, c, and e agrees with Robinson et al. (2004), however a moderate correlation between parameter a and d with lignin (r = -0.54) was also found. Although lignin is not bounded with cellulose (Jung and Ralph, 1990) the amount of lignin may influence the accessibility of microbes to substrate, and cell wall contents (Mandebvu et al., 1999), and consequently influence the amount of gas produced. The positive correlation between rain and digestibility (r = 0.37) agrees with Pitman and Holt (1982), who examined warm-season perennial grasses (Kleingrass 75 (*Panicum coloratum L.*), Kleingrass 75-25 (*Panicum coloratum L.*), green sprangletop [(*Leptochloa dubia* (H.B.K) Nees], and plains bristlegrass (*Setaria macrostachya* H.B.K.)). However, there was no correlation between humidity and digestibility; this is in contrary to the results reported by Pitman and Holt (1982). Those authors worked in 1978/79, since this time the weather in South Texas has become drier (Yu et al, 2006). They also worked with average data from different places in which the weather

Table 3.7: Pearson correlations among chemical and nutritional measurements and climatic conditions

Items [1]	a	b	c	d	e	Temperature	Dew point	Humidity	Wind speed	Rainfall	NDFD
DM	-0.13	0.08	0.21	0.08	0.17	-0.34	-0.24	0.27	-0.30	0.20	0.03
ADF	-0.58**	-0.20	-0.14	-0.55**	-0.23	-0.28	-0.33*	-0.10	0.34*	-0.35*	-0.58**
NDF	-0.49**	-0.31	-0.19	-0.59***	-0.12	-0.41*	-0.50**	-0.29	0.44**	-0.44**	-0.56**
Lignin	-0.54**	-0.21	-0.19	-0.54**	-0.25	-0.18	-0.23	-0.14	0.46**	-0.42**	-0.43**
EE	0.34*	0.35*	0.51**	0.42**	0.26	0.33*	0.42**	0.37*	-0.41*	0.50**	0.32*
Ash	0.14	-0.33*	0.05	-0.01	-0.34*	0.08	0.02	-0.20	-0.10	-0.15	0.27
CP	0.49**	0.19	0.18	0.49**	0.13	0.40*	0.46**	0.21	-0.34*	0.46**	0.58**
ADIN	0.29	0.14	-0.19	0.26	0.15	0.05	0.01	-0.26	0.20	-0.13	0.52**
NDIN	0.40*	0.24	-0.40	0.35*	0.20	0.19	0.20	-0.12	0.00	0.16	0.68***
Soluble CP	0.32	0.18	0.09	0.24	0.23	0.25	0.34	0.43*	-0.53	0.41	0.21
TDN_4											0.80***
TDN_6											0.84*
TDN_8											0.85*
TDN_{Weiss}											0.52

[1] a, b, c, d, and e are parameters of the two-pool logistic nonlinear function; EE = ether extract, % DM; TDN_4, TDN_6, and TDN_8 are TDN estimated at maintenance level of intake (TDN_{1x}) not adjusted for unavailable carbohydrate, assuming 4, 6, and 8 %/h passage rate; respectively; and TDN_{Weiss} is TDN_{1x} predicted by the Weiss et al. (1992) equation

* $P < 0.05$

** $P < 0.01$

*** $P < 0.001$

may be different. On the other hand, this study was conducted in one region in which the weather may have had a greater effect on digestibility. The relationship between temperature and CP was similar to that reported by Meyer and Brown (1985). During the rainy season, an increase in forage production occurs, and an increase in plant NDF and ADF was observed (Gonzales et al., 2005). Temperature has an important influence on plant CP concentration; a moderate correlation between CP and temperature (r = 0.40) and CP and rainfall (r = 0.46) was observed (Table 3.7), concurring with Yayneshet et al. (2009), who reported a significant relationship between CP and seasonality. Van Soest (1994) suggested that plants increase lignin to protect themselves against the wind resulting in a decline in nutritive value. Kloppenburg et al. (1995) reported a decrease in digestibility when temperature decreases working with irrigated pastures in New Mexico. In South Texas, there was a small positive correlation between digestibility and temperature (r = 0.24) was found. A small positive correlation between digestibility and temperature (r = 0.23) was also found, which is in contrast to Van Soest (1992), who examined temperate and tropical grasses all together. Temperate and tropical grasses may respond differently due to lower level of NDF in the temperate grasses (Van Soest, 1973) and differences in anatomy (Wilson and Hattersley, 1983).

In conclusion, these relationships between nutritive value and climate (Table 3.7) suggested that several factors affect plant quality and no single factor had complete control on digestibility. A combination of temperature, sunlight, rain, and nutrients available to the plant, are likely to dictate the quality of the forage.

Simulations of the ME and MP Balances

The accurate predictions of DMI and animal response under grazing systems with tropical grasses requires adequate measurements of NDF, lignin, CP, SP, and digestion rates for fiber and protein (Juarez Lagunes et al., 1999). Alison (1985) reported that if animals on rangeland could consume enough forage they could meet their requirements, although DMI is affected by animal and plant physical factors, and by plant-animal interactions. Sprinkle (1996) suggested that if not enough forage was available; no supplementation program will be helpful to achieve nutrient requirements.

The ME and MP balances are presented in Figure 3.2. For P1, the DMI predicted by the LRNS model for grazing Santa Gertrudis beef cows were not sufficient to meet the ME and MP requirements during all months except for April for MP. The average DMI (forage + supplement) predicted by the LRNS model was 1.75 ± 0.25% of the BW.

Similarly, for P2, the DMI predicted by the LRNS model was not sufficient to meet the ME and MP requirements during all period except for May for ME balance. The MP balance was negative during May, June, September, and October. This was very similar to P1. The average DMI (forage + supplement) predicted by the LRNS model was 1.86 ± 0.21% BW. In the same way for P3, the DMI predicted by LRNS model was not satisfactory to meet the energy during all year and protein requirements during all months except for July, August, and September for MP. The average DMI (forage + supplements) predicted by the LRNS model was 1.86 ± 0.21% of the BW for P3. For all three periods, cows calved in March and calves were weaned in October; thus, these results indicated the lack of ME and MP might have affected milk production and reproduction rates. Sprinkle (1996) reported

that the DMI necessary to meet maintenance requirements in beef cows consuming forage (TDN = 55%) was 2.6% of BW. This underprediction by the CNCPS has been reported in other experiments (Fox et al., 1992; Molina et al., 2004). Therefore, we simulated ME and MP balance using a DMI of 2.6% of BW (forage + supplement) and that cows were consuming on average 0.29%, 0.26%, and 0.22% of BW, respectively, of supplement for P1, P2, and P3. The DMI of forage was computed as the difference between total DMI and the supplement intake, and no forage substitution or increase in forage intake was assumed.

When 2.6% of BW was used to compute total DMI, the ME balances were negative except for August, September, March, and April; and the MP balance was negative for June, November, December, January, February, and March for P1. For P2, cows were deficient in ME excepted for May, June, and September and for MP during May, June, September, and October. During P3 cows were in deficit in ME excepted for July, August, and September, and for MP just during July, August, and September. Period 3 was during the intense dry period in South Texas and this may have influenced the nutritional requirements, forage availability, and consumption. Range animals can have their nutritional requirements altered by grazing activity, travel, and environmental stress (Allison, 1985). These results were different from the previous simulation and they were more consistent with the observation of cow performance as shown by the slight increase in BW (Figure 3.1) during the 3 periods. Figure 3.1 supports the hypothesis that cows had an overall positive MP and ME balance throughout the reproductive cycle because the average BW of the cows increased during these periods. Cows likely used the surplus of nutrients for growth and to deposit body reserves.

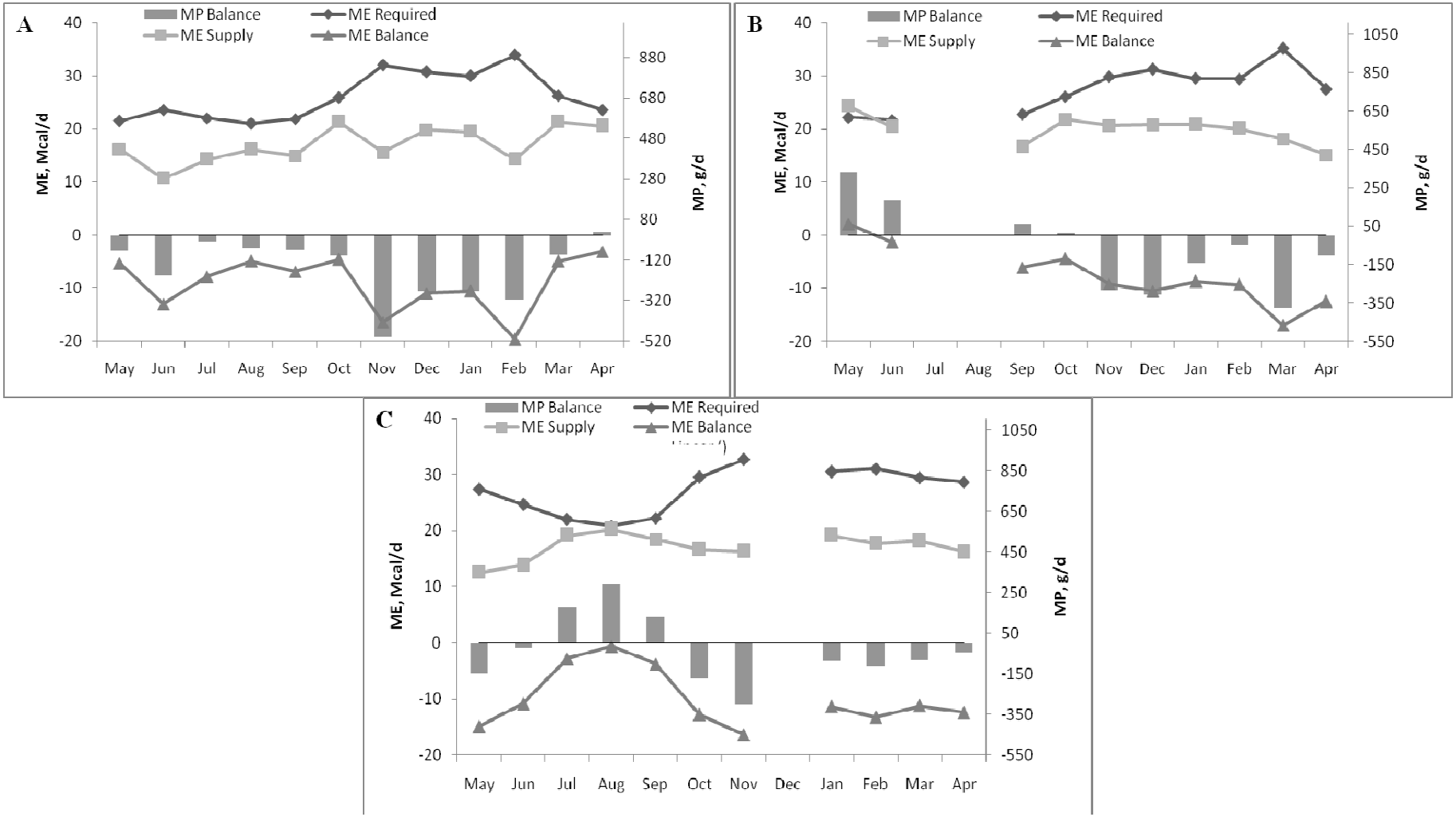

Figure 3.2. The ME and MP balances for May 2006 to Apr 2007 (A), May 2007 to Apr 2008 (B), and May 2008 to Apr 2009 as predicted by the Large Ruminant Nutrition System.

Winter- or spring-calving cows are usually in negative energy balance before calving due to the low intake of nutrients. May and June are likely to be when peak milk occurred for these Santa Gertrudis cows. Offering the necessary quantity and the proper supplementation to beef cows can improve the utilization of low quality forage (Ovenell et al. 1991). Reynoso-Campos et al. (2004) reported the onset of a negative energy balance on day 115 of lactation. The amount of nutrients necessary for positive energy balance was high and animals would have to eat large amounts of forage due to its low quality. However, in this case, the physical capacity of the rumen would be the first limiting factor. The poor quality of the forage and increase in MP and ME requirements during these months likely contributed to the negative protein and energy balance. According to Baumann et al. (2004), low-quality forage usually does not supply either energy or protein requirements to beef cows during early lactation. Winterholler et al. (2009) concluded that extra energy (0.75 kg/d of TDN) via supplement 60-d prepartum was not enough to avoid reduction in BW and BCS. Banta et al. (2008) reported a loss in BW when cows were fed with whole soybeans during mid to late gestation, and the authors reported a smaller gain in BW and BCS compared with a soybean meal/hulls supplement.

Juarez Lagunes et al. (1999) concluded that the LRNS model should improve prediction of nutrient requirements and animal performance, but nutrition models in general have to be used to predict animal requirements when only feed composition is available. The LRNS model is sensitive to forage chemical analysis and fermentation kinetics and accurate predictions of forage DMI are needed to adequately predict energy balance and supplementation strategies of grazing beef cows.

Implications

The use of in vitro fermentation data to estimate TDN is a valuable tool, but further work is needed in order to improve the predictions. The IVGP is a method that can be used to estimate degradation rates of feedstuffs and when combined with chemical analysis it can assist producers to improve animal productivity and make grazing management decisions. The LRNS model underpredicted DMI in grazing animals as per the observed performance of the animals. Using IVGP data can be used to estimate TDN values of warm season grasses, and degradation rates from different forage have to be used to calculated TDN values. Different forages, animal per se, and level of production have an effect on the passage rate, and different passage rate have to be used to estimate TDN value. The variation in the forage fermentation, and consequently TDN values, may affect animal performance (pregnancy and conception rates) that require close monitoring of forage quality and supplementation strategies to maintain level of production and profitability.

CHAPTER IV

EVALUATING THE STATISTICAL VARIATION IN PREDICTING DRY MATTER INTAKE OF GRAZING CATTLE USING THE N-ALKANE TECHNIQUE

Overview

The n-alkane technique has been widely used to determine DMI of grazing cattle. While there have been suggestions that increasing the number of samples per day decreases the error of the methodology and increases precision of estimating DMI, little information is available about what times of the day and number of days that fecal samples should be collected in order to reliably estimate DMI of grazing animals. The objectives of this study were to determine the variation structure within a day and across days when determining DMI using C_{32} alkane as an external marker; to determine the optimum fecal collection periods to estimate DMI; and to compare C_{31} and C_{33} as plant markers in computing DMI. Sixteen Brahman bulls stratified by previous RFI rankings were placed in 4 groups with 2 high and 2 low per groups. Groups were randomly assigned into 4 Coastal bermudagrass pastures [*Cynodon dactylon* (L.) Pers.] and stocked at a moderate to low grazing pressure. Corn gluten dosed with C_{32} n-alkane was used to estimate DMI. There were 3 periods (P1, P2, P3) of collection; each period was divided into 2 sub-periods in which fecal samples were collected twice daily (0700 and 1900 h) during the first 5 d and four times a day for the following 5 d, (0700, 1100, 1500 and 1900 h). Gas chromatography was used to determine n-alkanes in the forage and fecal samples. The concentration of C_{31} was less than the C_{33} in the forage for all periods (P <0.0001), but the concentration of C_{31} and C_{33} in feces was not different. The average concentrations of C_{32} alkane in the forages were 5.1, 7.6, and 9.6

mg/kg DM, for P1, P2, and P3; respectively, with an average of 7.5 mg/kg DM for all periods. During P1 and P2, the prediction of DMI using C_{33} had a better fit (smaller -2×Log and AIC) than C_{31} either with or without adjustments for forage C_{32}. The variation in DMI decreased when adjustments for forage C_{32} was not used. The variances of DMI were similar using C_{31} across days, but the correlations between days were low, suggesting that several days of collection were needed to accurately predict DMI. Correlations between times were medium to high for all periods and varied from 0.65 to 0.97 for C_{31} and from 0.26 to 0.96 for C_{33}. When all periods were analyzed together, estimates of DMI either using C_{31} or C_{33} had low correlations between days of collection. In addition, the adjustment for forage C_{32} did not improve the variance and (co)variance matrix. In conclusion, C_{33}/C_{32} had the lowest variation in predicting DMI and at least 5 d of fecal collection were needed to decrease the variability of DMI. The optimum times for fecal collection were 0700 and 1500 h and it was important to adjust for C_{32} alkane concentration to predict DMI of Brahman bulls grazing Coastal bermudagrass.

Introduction

According to Dove and Mayes (1991), an ideal marker should not be digested in the digestive tract, should pass at the same rate as the digesta, and must not affect or be affected by the gastrointestinal tract and/or microbial population (Giráldez, 2006). Using alkanes to predict DMI was initially proposed by Mayes and Lamb (1984). They suggested the possibility of using n-alkanes as an indigestible marker because n-alkanes are more chemically inert and easier to analyze compared to long-chained fatty acids. Vulich et al.

(1995) pointed out that in order to determine DMI the methodology requires the use of ratios of n-alkanes in the feces. Currently, markers that have been widely used to predict DMI for grazing cattle are hydrocarbons of plant cuticular wax (odd-chained n-alkanes) in the forage and dosing with even-chained n-alkanes which are not in the forage (Bovolenta et al. 1994; Hameleers and Mayes, 1998). Dosed n-alkanes have a higher recovery compared to naturally occurring alkanes (Mayes et al., 1986), and the n-alkanes fecal recovery increases with increasing carbon chain length (Mayes et al. 1986; Bovolenta et al., 1994). The n-alkanes present in herbage species are odd-chain, and vary from C_{25} to C_{35}.The most abundant n-alkanes that are present in the herbage are C_{29}, C_{31}, and C_{33} (Mayes et al., 1984). Thus, we analyzed for C_{31} and C_{33}.

Dove and Mayes (2006) in agreement with Mayes et al. (1986), and Bovolenta et al., (1994), pointed out that octacosane (C_{28}) and dotriacontane (C_{32}) can be used as external markers due to simple and inexpensive access and their low concentration in the forage. These authors also concluded that n-alkanes can be used to estimate DMI without measuring complete fecal output. According to Vulich et al. (1991), the n-alkane technique does not require knowledge of digestibility of the forage in order to be used in a large scale experiment. Malossini et al. (1996) concluded that increasing the number of samples per day decreases the error of the methodology and increases precision of estimating DMI. However, little information is available about what time of the day and for how many days that fecal samples should be collected in order to reliably determine DMI of grazing animals.

The objectives of this study were (1) to determine the variation structure within a day and across days when determining DMI using C_{32} and C_{33} n-alkanes; (2) to determine the

optimum collection period for fecal material to determine DMI; and (3) to compare C_{31} and C_{33} as markers in computing DMI.

Material and Methods

The study was conducted at the Texas AgriLife Research Center in Overton, TX, in humid east Texas (32°16´N 94°59´W, average rainfall 88.9 mm, mean temperature 27.6°C) during the summer of 2008. Purebred Brahman bulls (n=16) stratified by previous RFI rankings were placed in 4 groups with 2 high and 2 low per groups with an average age of 18 months were randomly allotted into 4 Coastal bermudagrass pastures [*Cynodon dactylon* (L.) Pers.], and were stocked at a moderate to low grazing pressure. The average forage mass during the study period was 6803 kg/ha.

Preparation of the Marker

Corn gluten pellets were prepared at the Texas AgriLife Research Center in Uvalde, TX, and used as the carrier for C_{32} n-alkane. Corn gluten pellets were sieved using a 2-mm sieve to remove fines and 400 ± 1 g was weighed and placed in a paper bag. On the day of preparation, the labeled corn gluten was transferred to a 760-ml Rubbermaid container and placed in an oven at 75ºC for approximately 2 h. Using a 30-ml Minipet Pipettor (VWR, cat # 54848-204), 10 ml of C_{32}-alkane solution was slowly pipetted over the warm corn gluten. The solution was composed of 7 g of C_{32} (Dotriacontane, Aldrich cat # D22, 310-7) in 350 ml heptane (VWR cat # EM-HX0080-6) and heated on low temperature until a solution was formed. After each set was prepared, warm heptane was used to clean the pipette. After

adding the solution over the corn gluten, samples were placed at room temperature to allow the heptane to evaporate for approximately 30 min, and samples were placed in a 75ºC oven for approximately 1 h. Samples were them placed in paper bags and labeled for each trial. One sample of each set was taken for future standard analysis.

Feeding and Feces Collection Procedures

After one week of adaptation to corn gluten fed via Calan gate units in the pastures, bulls which had been previously trained to eat in Calan gates, were individually fed 400 g of corn gluten two times per day (0700 and 1900 h). Following the adaptation period, bulls were fed corn gluten (400 g) labeled with 200 mg C_{32} n-alkane solution samples twice daily (0700 and 1900 h) in order to reduce diurnal variation as suggested by Smit et al. (2005). During the first 5 d of the trial, fecal samples were collected twice daily (0700 and 1900 h) and during the following 5 d of the period, fecal samples were collected four times daily (0700, 1100, 1500 and 1900 h). Fecal samples were collected immediately upon defecation or via rectal palpation and placed in zip lock bags. After fecal samples were collected they were placed in a -20 ^{o}C freezer for 24 h. The frozen samples were placed in a 60 ºC oven for 72 h. Forage samples selected to represent the grazed strata were collected daily beginning 2 d prior to the start of dosing of n-alkanes. In order to analyze the difference in n-alkane concentration between different parts of the plant, a leaf/stem separation was performed. There were 3 periods of collections: period 1 (**P1**), period 2 (**P2**), and period 3 (**P3**) interchanged with adaptations periods as shown in Figure 4.1.

Period 1			Period 2			Period 3	
5 days of adaptation	5 days of collection, 4X/day	**Interval** 4 days	5 days of adaptation	5 days of Collection, 4X/day	**Interval** 18 days	5 days of adaptation	5 days of collection, 4X/day

Figure 4.1: Collections fecal design of the experimental periods

Chemical Analyses. All forage and fecal samples were dried at 60 ºC and ground using a cyclone mill fitted with a 1 mm screen, prior to extraction and subsequent gas chromatography. All forage samples were sent to Cumberland Valley Analytical Services (Cumberland Valley Analytical Services, Inc.; http://www.foragelab.com/) for the following analyses: DM was performed in two steps; the first step was according to Goering and Van Soest (1970), and during the second step oven temperature was increase to 105 ºC, according to the National Forage Testing Association (2002). Ash was performed according to AOAC (2002, method 942.05), CP, and non-sequential ADF analyses were performed according to AOAC (2002; method 2001.11 and 973.18*;* respectively). The NDF was determined according to Van Soest et al. (1991). The ether extract (**EE**) was determined by AOAC (2002; method 920.39). The lignin analysis was performed according to Goering and Van Soest (1970) using 72% sulfuric acid with modifications. The corn gluten was ground using a cyclone mill fitted with a 1-mm screen.

Alkane Determination

In order to determine n-alkanes in the fecal and forage samples and in the corn gluten, a gas chromatography system (Agilent 6890N, Santa Clara, CA, USA) with auto

sampler and computer program was used. A Supelco Special Order SPB-1, fused silica capillary column, 30 x 0.75mm ID x 1.00 um was used. For each run, 5 standard samples were included for calibration. The injector was set to add 1.0 µl of sample in a split ratio of 4.3:1 and washed with heptanes at both pre- and post-injection. The oven temperature was set at 285 ºC and held for 12 min, and the detector heater was set at 320 ºC using a gradient run. Initial temperature was set at 210 ºC, temperature ramped to 285 ºC at 25 ºC/minute and held for eight minutes, then ramped to 310 ºC at 25 ºC/min and held for 2 min. The injector temperature was set at 300 ºC and the detector temperature was at 320 ºC.

Experimental Design

The experiment was designed as double repeated measurements (5 days of fecal collection and 4 times of fecal collection within a day) in a completely randomized block design (3 periods as blocks) (SAS date Inst., Cary, NC). Animals were the experimental unit and they were maintained in the same pasture during the different periods to maintain the established hierarchy within a pasture. Two statistical analysis procedures were performed to understand the variance and (co)variance (**var-(co)var**) structure of days and times of collection for both C_{31} and C_{33} with and without adjustments for forage C_{32} across periods (SAS date Inst., Cary, NC). The first statistical model was performed for each period independently, as follows:

$$Y_{ijklm} = \mu + R_i + T_j + D_k + R\times T_{ij} + R\times D_{ik} + T\times D_{jk} + R\times T\times D_{ijk} + A_{il} + P_m + \varepsilon_{ijklm} \quad \text{Eq. [5]}$$

Where Y is the observed variable; µ is the overall mean; R is the fixed effect of RFI group; T is the fixed effect of time of fecal collection within a day; D is the fixed effect of day of

collection; A is the random animal effect associated with RFI; P is the random effect of pasture; and ε is the identical, independent, and normally distributed random error with N~(0, σ^2).

The second statistical model was done with all periods together as shown below:

$$Y_{ijklm} = \mu + R_i + T_j + D_k + R\times T_{ij} + R\times D_{ik} + T\times D_{jk} + R\times T\times D_{ijk} + A_{il} + P_m + I_n + \varepsilon_{ijklmi} \quad \text{Eq. [6]}$$

where Y is the observed variable; μ is the overall mean; R is the fixed effect of RFI group; T is the fixed effect of time of fecal collection within a day; D is the fixed effect of day of collection; A is the random animal effect associated with RFI; P is the random effect of pasture; I is the random effect of period; and ε is the identical, independent, and normally distributed random error with N~(0, σ^2).

All statistical analyses were performed using PROC MIXED of SAS, and the goodness-of-fit for prediction of DMI was accessed with the -2×Log and the Akaike's Information Criteria (**AIC**) statistics (SAS Inst., Cary, NC).

Because two levels of repeated measures were evaluated (days and times of collection), the var-(co)var structure for un@un (unstructured and unstructured) and un@ar(1) (unstructured and autoregressive first degree) were used. The calculation of var-(co)var assuming un@ar(1) structure (Gao et al., 2006) is shown below with the following scheme of var-(co)var for 2 and 3 levels, respectively for factors 1 and 2.

$$UN \,@\, AR(1) = \begin{bmatrix} \sigma_1^2 & \sigma_{21} \\ \sigma_{21} & \sigma_2^2 \end{bmatrix} \otimes \begin{bmatrix} 1 & \rho & \rho^2 \\ \rho & 1 & \rho \\ \rho^2 & \rho & 1 \end{bmatrix} = \begin{bmatrix} \sigma_1^2 & \sigma_1^2\rho & \sigma_1^2\rho^2 & \sigma_{21} & \sigma_{21}\rho & \sigma_{21}\rho^2 \\ \sigma_1^2\rho & \sigma_1^2 & \sigma_1^2\rho & \sigma_{21}\rho & \sigma_{21} & \sigma_{21}\rho \\ \sigma_1^2\rho^2 & \sigma_1^2\rho & \sigma_1^2 & \sigma_{21}\rho^2 & \sigma_{21}\rho & \sigma_{21} \\ \sigma_{21} & \sigma_{21}\rho & \sigma_{21}\rho^2 & \sigma_2^2 & \sigma_2^2\rho & \sigma_2^2\rho^2 \\ \sigma_{21}\rho & \sigma_{21} & \sigma_{21}\rho & \sigma_2^2\rho & \sigma_2^2 & \sigma_2^2\rho \\ \sigma_{21}\rho^2 & \sigma_{21}\rho & \sigma_{21} & \sigma_2^2\rho^2 & \sigma_2^2\rho & \sigma_2^2 \end{bmatrix}$$

Therefore, the var-(co)var structure for the ar(1) factor depends on the un var-(co)var values to compute the final variance for each level combination of both repeated measures. When un@un or un@ar(1) structures were used, the individual un and ar(1) matrices will be shown.

Results and Discussion

The concentration of C_{31} in the bermudagrass was less than the concentration of n-alkane C_{33} for all periods (P <0.0001). However, the concentration in the feces was not different between C_{31} and C_{33}. Mayes et al. (1986) reported higher values for C_{31} in perennial ryegrass. In addition, Smith and Strickland (2007) showed a higher value for C_{31} in annual ryegrass. According to Oliveira and Salatino (2000), n-alkane concentration in the forage may be influenced by high light, low air humidity, and high temperatures that can increase the alkane wax production. These environmental factors may have impacted the concentration of C_{31} and C_{33} of bermudagrass samples during the summer months of July-August.

Variance and Correlation Analysis

Analysis by periods: Table 4.1 presents the variance and correlation (**var-cor**) analysis of the goodness-of-fit of the DMI using C_{31} and C_{33} with or without adjustments for forage C_{32}, and assuming two levels of repeated measure design (day and time, and time and day). Except for P3, the prediction of DMI using C_{33} had a better fit (smaller -2×Log and AIC) than C_{31} either with or without adjustments for forage C_{32}. This result was in agreement with Mayes and Lamb (1984) and Mayes et al. (1986), who have reported an improvement in fecal recovery as chain length increases and consequently decreases the variation. Molina et al. (2004) determined DMI of lactating cows using capsules with n-alkane and reported less DMI variation when using C_{33} compared to C_{31}. Similarly, Mayes et al. (1986) using lambs fed with ryegrass hay at different levels of DMI, and ryegrass hay plus concentrate (barley, sugar beet pulp, soybean meal, and white fish meal) comparing C_{31} and C_{33}, as the marker and Vulich et al (1991) feeding lambs ad libitum with *Lolium perenne*, and Poa and Festuca species, comparing C_{29}, C_{31}, and C_{33}, as the marker, both authors selected C_{33} to determine DMI. Malossini et al. (1996) working with mid-lactation cows using paper soaked with C_{32} as a marker-carrier found a lower variability when C_{33} was used to calculate forage DMI compared to C_{31}. When forage C_{32} was not used to compute DMI, the variation decreased for all cases (Table 4.1). This hypothesis supports the idea that the variation of alkanes among days and variation among times in the feces may have an effect in the analysis.

Lippke (2002) conducted a review and concluded that the C_{32}/C_{33} pair should be used to estimate forage DMI. Berry et al., (2000) working with dairy cows dosed with controlled-

release capsules, releasing 388.2 mg /d of C_{32}, reported a smaller inconsistency in DMI predicted by the C_{33}/C_{32} pair when compared to the C_{31}/C_{32} pair.

Tables 4.2 and 4.3 have the var-(co)var structures and values of DMI for individual and combined periods of collections. When the DMI was predicted using C_{31} without adjustment for forage C_{32} (Table 4.2), first-order autoregressive var-(co)var structure was observed for days of collection and unstructured var-(co)var was observed for times of collection. For C_{33} (Table 4.2), the var-(co)var was unstructured for days and times of collection. A similar outcome was observed when DMI was predicted either using C_{31} or C_{33} with adjustment for forage C_{32} (Table 4.3). A first-order autoregressive var-(co)var indicated a lower correlation that the further the levels were apart from each other, but with the same variance. In the case of unstructured var-(co)var, variance and correlation among levels of a factor (e.g. days of collection) may change without a defined pattern.

Even though the variances of DMI using C_{31} without adjustment for forage C_{32} (Table 4.2) were similar across days, the correlation among different days of collection was either very low or naught, suggesting that days of collection yielded completely different estimates of DMI and therefore several days of collection were necessary to accurately estimate DMI. A similar outcome was obtained when DMI was computed with adjustments for forage C_{32} content of the forage.

Table 4.1: Selection of the best variance-(co)variance matrix structure with two levels of repeated measured variables (time and day) and two sequences (time – day and day – time) for individual or combined periods 1, 2, and 3, using C_{31} and C_{33} with and without adjustments for forage C_{32}

Matrix Structures	Periods							
	1		2		3		1, 2, and 3	
	-2×Log	AIC	-2×Log	AIC	-2×Log	AIC	-2×Log	AIC
Day-Time	C_{31} without forage C_{32} adjustment							
un@un	---	---	---	---	817.5	867.5	3146.7	3198.7
un@cs	---	---	---	---	---	---	---	---
un@ar(1)	---	---	---	---	---	---	---	---
Time-Day								
un@un	---	---	---	---	---	---	---	---
un@cs	995.6	1019.6	1015.4	1039.4	843.7	867.7	3215.8	3241.8
un@ar(1)	997.8	1021.8	---	---	838.3	862.3	3228.9	3254.9
Day-Time	C_{33} without forage C_{32} adjustment							
un@un	592.5	642.5	707.5	757.5	---	---	2363.9	2415.9
un@cs	648.4	682.4	---	---	---	---	2492	2528
un@ar(1)	---	---	---	---	---	---	2422.2	2448.2
Time-Day								
un@un	---	---	707.5	757.5	---	---	2363.9	2415.9
un@cs	649.6	673.6	772.2	796.2	714.5	738.5	2414.3	2440.3
un@ar(1)	651.5	675.5	---	---	713.4	737.4	2422.2	2448.2
Day-Time	C_{31} with forage C_{32} adjustment							
un@un	---	---	---	---	---	---	---	---
un@cs	935.3	967.3	---	---	---	---	---	---
un@ar(1)	---	---	---	---	---	---	2956.6	2982.6
Time-Day								
un@un	---	---	---	---	---	---	---	---
un@cs	962.1	986.1	911.7	935.7	671.3	695.3	2943.5	2969.5
un@ar(1)	963.6	987.6	912.1	936.1	667.4	691.4	2956.6	2982.6
Day-Time	C_{33} with forage C_{32} adjustment							
un@un	506.5	556.5	607	657	499.4	549.4	1972.2	2024.2
un@cs	578.2	612.2	---	---	---	---	2158.9	2194.9
un@ar(1)	---	---	---	---	---	---	---	---
Time-Day								
un@un	506.5	556.5	607	657	499.4	549.4	1972.2	2024.2
un@cs	556.7	580.7	666.3	690.3	547	571	2028.1	2054.1
un@ar(1)	560	584	---	---	548.3	572.3	2041.6	2067.6

Table 4.2: Variance (diagonal) and correlation matrices for selected days (D) and times (T) of fecal collection to estimate DMI using C_{31} and C_{33} without adjustments for forage C_{32} for individual and combined periods 1, 2, and 3 [1]

Periods																					
1						2						3						1, 2, and 3				
--- C_{31} ---																					
Days: ar(1)						Days: ar(1)						Days: ar(1)						Days: un				
	1	2	3	4	5		1	2	3	4	5		1	2	3	4	5		1	2	3	4	5
1	1.00					1	1.00					1	1.00					1	1.00				
2	0.01	1.00				2	0.02	1.00				2	0.38	1.00				2	0.26	1.02			
3	0.00	0.01	1.00			3	0.02	0.02	1.00			3	0.14	0.38	1.00			3	0.18	0.32	0.38		
4	0.00	0.00	0.01	1.00		4	0.02	0.02	0.02	1.00		4	0.05	0.14	0.38	1.00		4	0.18	0.17	0.15	1.40	
5	0.00	0.00	0.00	0.01	1.00	5	0.02	0.02	0.02	0.02	1.00	5	0.02	0.05	0.14	0.38	1.00	5	0.09	0.27	0.09	0.04	0.48
Times: un						Times: un						Times: un						Times: un					
	1	2	3	4			1	2	3	4			1	2	3	4			1	2	3	4	
1	3.53					1	6.89					1	1.53					1	6.56				
2	0.88	2.81				2	0.96	10.08				2	0.80	1.60				2	0.92	6.95			
3	0.80	0.86	3.82			3	0.90	0.97	12.41			3	0.78	0.96	1.76			3	0.88	0.96	8.29		
4	0.65	0.73	0.79	3.10		4	0.92	0.97	0.97	13.1		4	0.70	0.85	0.86	1.77		4	0.83	0.92	0.93	8.32	
--- C_{33} ---																							
Days: un						Days: un						Days: un						Days: un					
	1	2	3	4	5		1	2	3	4	5		1	2	3	4	5		1	2	3	4	5
1	1.00					1	3.00					1	1.31					1	2.49				
2	0.07	0.51				2	-0.4	0.69				2	0.33	0.67				2	0.25	1.41			
3	0.24	0.43	0.18			3	0.01	0.00	1.11			3	0.02	0.23	1.11			3	0.20	0.31	1.15		
4	0.17	0.29	0.22	0.54		4	0.54	-0.07	-0.11	4.78		4	0.22	0.10	0.50	0.53		4	0.56	0.08	0.10	2.79	
5	0.00	0.37	0.18	0	0.28	5	0.12	0.28	-0.24	-0.04	1.92	5	0.13	0.56	0.37	-0.1	1.38	5	0.13	0.38	0.16	0.02	1.49
Times: un						Times: un						Times: un						Times: un					
	1	2	3	4			1	2	3	4			1	2	3	4			1	2	3	4	
1	1.21					1	1.00					1	1.00					1	1.00				
2	0.78	1.30				2	0.94	1.28				2	0.80	0.78				2	0.89	1.04			
3	0.66	0.85	1.42			3	0.88	0.96	1.44			3	0.73	0.93	0.70			3	0.84	0.95	1.20		
4	0.26	0.60	0.70	1.34		4	0.87	0.95	0.95	1.45		4	0.63	0.80	0.76	0.83		4	0.78	0.90	0.92	1.22	

[1] ar(1) and un are first-order autoregressive and unstructured variance and (co)variance structures, respectively. Times 1, 2, 3, and 4 are fecal collections at 0700, 1100, 1500, and 1900 h, respectively, within a day. These are symmetrical matrices; therefore, the top values are identical to the values shown in the bottom part within a matrix

Table 4.3: Variance (diagonal) and correlation matrices for selected days (D) and times (T) of fecal collection to estimate DMI using C_{31} and C_{33} with adjustments for forage C_{32} for individual and combined periods 1, 2, and 3 [1]

Periods																					
1						2					3						1, 2, and 3				
--- C_{31} ---																					
Days: ar(1)						Days: ar(1)					Days: ar(1)						Days: un				
	1	2	3	4	5		1	2	3	4	5		1	2	3	4	5		1	2	3	4	5
1	1.0					1	1.0					1	1.0					1	1.0				
2	0.07	1.0				2	0.01	1.0				2	0.35	1.0				2	0.24	1.06			
3	0.01	0.07	1.0			3	0.00	0.01	1.0			3	0.12	0.35	1.0			3	0.18	0.31	0.44		
4	0.00	0.01	0.07	1.0		4	0.00	0.00	0.01	1.0		4	0.04	0.12	0.35	1.0		4	0.11	0.15	0.17	1.36	
5	0.00	0.00	0.01	0.07	1.0	5	0.00	0.00	0.00	0.01	1.0	5	0.02	0.04	0.12	0.35	1.0	5	0.07	0.21	0.08	0.09	0.50
Times: un						Times: un						Times: un						Times: un					
	1	2	3	4			1	2	3	4			1	2	3	4			1	2	3	4	
1	4.05					1	7.40					1	2.04					1	7.18				
2	0.86	3.11				2	0.94	11.8				2	0.75	2.28				2	0.89	7.85			
3	0.81	0.87	3.92			3	0.88	0.96	14.2			3	0.69	0.91	2.43			3	0.85	0.99	9.24		
4	0.62	0.70	0.74	3.30		4	0.90	0.96	0.96	15.0		4	0.65	0.82	0.83	2.44		4	0.80	0.90	0.92	9.25	
--- C_{33} ---																					
Days: un						Days: un						Days: un						Days: un					
	1	2	3	4	5		1	2	3	4	5		1	2	3	4	5		1	2	3	4	5
1	1.0					1	3.12					1	1.32					1	2.54				
2	0.08	0.54				2	-0.3	0.64				2	0.26	0.97				2	0.27	1.68			
3	0.25	0.44	0.16			3	0.08	0.01	1.25			3	0.03	0.24	1.31			3	0.21	0.32	1.48		
4	0.06	0.27	0.12	0.63		4	0.28	-0.11	-0.13	5.33		4	0.28	0	0.52	0.97		4	0.42	0.05	0.12	3.41	
5	0.03	0.37	0.23	-0.2	0.3	5	0.06	0.26	-0.27	-0.01	2.67	5	0	0.40	0.39	-0.1	1.1	5	0.10	0.27	0.16	0.08	1.84
Times: un						Times: un						Times: un						Times: un					
	1	2	3	4			1	2	3	4			1	2	3	4			1	2	3	4	
1	1.25					1	1.00					1	1.00					1	1.00				
2	0.76	1.45				2	0.92	1.38				2	0.79	0.63				2	0.86	1.05			
3	0.64	0.81	1.81			3	0.85	0.94	1.48			3	0.58	1.01	0.80			3	0.79	0.93	1.22		
4	0.23	0.58	0.71	1.53		4	0.84	0.94	0.93	1.51		4	0.50	0.86	0.67	1.00		4	0.74	0.88	0.89	1.21	

[1] ar(1) and un are first-order autoregressive and unstructured variance and (co)variance structures, respectively. Times 1, 2, 3, and 4 are fecal collections at 0700, 1100, 1500, and 1900 h, respectively, within a day. These are symmetrical matrices; therefore, the top values are identical to the values shown in the bottom part within a matrix

On the contrary, estimates of DMI across times of collection were medium to highly correlated for all periods regardless of adjustments for forage C_{32} (Tables 4.2 and 4.3), and they tended to decrease with time as expected. Without adjustment for forage C_{32} content (Table 4.2), correlation varied from 0.65 to 0. 97 for C_{31} and 0.26 to 0.96 for C_{33}, but a smaller variance was observed for estimates of DMI using C_{33} (0.70 to 1.30) than C_{31} (1.53 to 12.41; Table 4.2). Similar results were found when forage C_{32} was used to adjust the predicted DMI. Period 3 had the least variance for all times of collection compared to P1 and P2 (Tables 4.2 and 4.3).

All periods. When all periods were analyzed together, unstructured var-(co)var was the best fit for both days and times of fecal collection in determining DMI (Tables 4.2 and 4.3). Similar to the individual periods, estimates of DMI either using C_{31} or C_{33} had low correlations between days of collection (Table 4.2) and the variance varied from 0.38 to 1.40 kg^2/d^2. Adjusting for forage C_{32} did not improve the var-cor matrix values either (Table 4.3). The variance tended to be greater with C_{33} than with C_{31} to estimate DMI regardless of the adjustment with forage C_{32} (Tables 4.2 and 4.3). These results were in agreement with the individual analysis of periods in which C_{31} yields lower variance than $C_{33,}$ and that at least 5 d were needed to accurately estimate DMI using alkanes because of the lack of (lower) correlation among days of collection.

These results indicated that at least 5 d were needed to estimate DMI using alkanes and that time of collection were highly correlated. Therefore, fewer collections within a day may be adequate to estimate DMI, such as at 0700 and 1500 h. The estimates of DMI using C_{33} for times of collection within days had less variance than C_{31}, but there was indication

that C_{31} had the least variance across days of collection. Mayes et al. (1986) stated that if the variation between morning and afternoon DMI prediction were less than 5%, one collection per day was enough to estimate DMI. Otherwise, at least two collections within a day were needed to have a reasonable value for DMI. One reason for that variation can be explained by diurnal variation, has been shown by different authors (Mayes et al. 1986).

Ferreira et al. (2004) working with non-lactating cows dosed with controlled-release capsules containing n-alkane releasing (317.2 mg/d of C_{32}) and eating hay twice daily, found no difference in fecal sampling between 0800 and 2000h. Olivan et al. (2007) working with beef cattle dosed once a day with paper pellets containing C_{24}, C_{32}, and C_{36}, and collecting fecal samples 3 times a day with 8 h interval, concluded that one sample per day, with a 24 h interval, was sufficient to estimate DMI. These results support our conclusions of high correlation across times of collection within a day in which one or two collections within a day are needed to estimate DMI.

The least variable time of collection in this study was at 0700 for both C_{31} and C_{33} regardless of the adjustment for forage C_{32}. This can be explained by the grazing behavior of the animals, that usually grazing early in the morning and late in the afternoon. Mann and Stewart (2003) working with yearling bulls (Hereford and Holstein-Friesland), determined DMI using daily cut warm season perennial grass, kikuyu grass (*Pennisetum clandestinum* Hochst.)**,** in Calan gates, reported a difference of 18% in DMI between morning and afternoon collections. The morning collection underestimated DMI and the afternoon collection overestimated DMI. Authors indicated that one reason for this variation was likely because animals were dosed orally once a day. This variation may have been avoided if

animals were dosed twice a day (a 12 h period interval) to keep the constant flow in digestive tract (Mann and Stewart, 2003). Even though Mayes et al. (1986) reported that animals dosed once a day should be enough to estimate DMI.

Fecal and Forage Recovery

Fecal recovery did not differ between C_{31}, C_{32} and C_{33} for P1 (110.2 ± 17.9, 126.7 ± 23.6, and 113.5 ± 24.0 mg/kg DM), respectively. This was in agreement with Dove et al. (2002), who worked with controlled-release capsules and reported no differences between C_{31} and C_{32} recovery in fecal samples. The higher recovery for C_{32} can be supported by the association of the liquid phase of the digesta and dosed n-alkanes because the natural alkane associates with the solid part of the digesta (Mayes et al., 1986). Several (Hendricksen, 2002; Oliván, 2007; Dove et al., 2002) have reported a higher value for dosed alkanes. However, the recovery for P2 (189.9 ± 34.1, 107.4 ± 25.9, and 174.9 ± 32.0 mg/kg DM), and P3 (116.5 ± 26.2, 75.5 ± 12.5, and 123.2 ± 31.5 mg/kg DM), respectively for C_{31}, C_{32}, and C_{33}, the average for all periods did differ between C_{31} and C_{32}; and C_{33} and C_{32} (139.1 ± 45.1, 103.4 ± 30.1, and 137.7 ± 39.9 mg/kg DM), respectively. Ferreira et al. (2004) reported a higher concentration of C_{31} compared to C_{33}.

Casson et al. (1990) proposed that the minimum concentration of n-alkane in forages should be 50 mg/kg DM in odd numbered carbons. For P1, the recoveries for C_{31} and C_{33} were 42.3 ± 7.2 and 78.5 ± 9.4 mg/kg DM, respectively. For P2, recoveries were 86.0 ± 21.1 and 113.3 ± 27.5, respectively for C_{31} and C_{33}; and for P3, the recoveries increased to 95.6 ± 16.2 and 125.3 ± 18.7 for C_{31} and C_{33}, respectively. Valient et al. (2003) pointed out that although those values were suggested by Casson et al. (1990) and Laredo et al. (1991), there

was no research to support the idea that a minimum of 50 mg/kg DM was required to have a precise analysis. There were no differences with values lower than 50 mg/kg DM to accurately estimate DMI, diet composition, and digestibility under mixed concentrate and forage diets (Valient et al. 2003). The authors also suggested that this value should be ignored. The same recovery rate was found by Berry et al. (2000) for C_{33} and C_{32}; however they found a lower recovery for C_{31}.

The average concentrations of C_{32} alkane in bermudagrass samples were 5.1 ± 1.0, 7.6 ± 2.1, and 9.6 ± 1.4 mg/kg DM, respectively, for P1, P2, and P3, with a 3 period average of 7.5 ± 2.4 mg/kg DM. Interesting, the concentration of C_{32} increased during the periods. Smit et al. (2005) suggested that a change in concentration of C_{32} had an influence in predicted DMI values. The average concentrations for 3 n-alkanes are present in Table 4.4.

Table 4.4: Concentration (mg/g) of n-alkanes present in the leaf and stem of bermudagrass for all periods

Items	Periods							
	1		2		3		1, 2, and 3	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
C_{31}								
Leaf	0.055	0.014	0.058	0.007	0.074	0.009	0.062	0.013
Stem	0.033	0.005	0.063	0.008	0.068	0.016	0.055	0.019
C_{32}								
Leaf	0.006	0.001	0.004	0.002	0.008	0.001	0.006	0.002
Stem	0.005	0.000	0.006	0.000	0.007	0.001	0.006	0.001
C_{33}								
Leaf	0.054	0.018	0.057	0.024	0.065	0.016	0.059	0.059
Stem	0.116	0.020	0.133	0.021	0.125	0.021	0.125	0.125

There were low concentrations of C_{32} in the leaf and stem during all periods (Table 4.4). The C_{33} present in the stems had the greatest values when compared to C_{31}. However, the concentration in the leaf was the same.

Implications

In conclusion, the best times to collect fecal samples in order to predict DMI in grazing animals were early in the day and late in the evening (e.g. 0700 and 1900), and multiple days were necessary to obtain correct values. Thus, it is recommended that fecal collections be made during five consecutive days. The 1500-h collection is recommended due to the fact that later collections become cost prohibitive if fecal collections are made over extended periods. Dry matter intake using the C_{33}/C_{32} pair had the lowest variation compared to the C_{31}/C_{32} pair. Without adjustments for C_{32} the variation decreases, however it is important to use C_{32} values to predict DMI due the increasing concentration of C_{32} in the bermudagrass during the periods.

CHAPTER V

PREDICTION OF DRY MATTER INTAKE ON BERMUDAGRASS USING N-ALKANES AND BRAHMAN BULLS WITH RANKINGS FOR RESIDUAL FEED INTAKE

Overview

The objectives of this study were to compare different n-alkanes times of fecal collections to estimate DMI, and to evaluate the previously calculated ranking residual feed intake conditions (**RFI_c**) and subsequently in grazing condition (**RFI_g**) on Coastal bermudagrass [*Cynodon dactylon* (L.) Pers.] pastures. Purebred Brahman bulls, previously phenotyped in dry lot as either efficient (n = 8, low RFI_c) or inefficient (n =8, high RFI_c), were allotted among 4 pastures (2 low and 2 high per pasture) of Coastal bermudagrass. Corn gluten labeled with C_{32} was offered to animals, as a marker carrier. Fecal samples were collected 4 times daily (morning: 0700, 1100, and afternoon: 1500, and 1900 h) and were collected in 3 periods during 5 days in each period. Gas chromatography was used to analyze the n-alkane concentration of the forage (C_{32}) and fecal (C_{31} and C_{33}) samples. Four methods were used to estimate DMI: C_{31} or C_{33} with or without adjustment for C_{32} (C_{31_0} and C_{33_0}, respectively). Within a method, treatments (**TRT**) were assumed to be either individual or combination of different times of daily fecal collection. Statistical analyses included the fixed effects of TRT and RFI_c, and the random effects of period, days within period, animal, and pasture. The statistical model that had the least Akaike's Information Criteria to predict DMI was obtained with C_{31_0} (8199.6) followed by C_{33} (8661.4). The predicted DMI using C_{31}, C_{33}, C_{31_0}, and C_{33_0} alone or in combination (C_{31} and C_{33}, or C_{31_0} and C_{33_0}) were different (*P* =

0.0106). There was a difference between morning and afternoon fecal collections on the predicted DMI using C_{31} ($P = 0.0010$), C_{33} ($P = 0.0001$), C_{31_0} ($P = 0.0010$), or C_{33_0} ($P < 0.0001$). There was a significant difference ($P = 0.0188$) in the mean BW (459 and 409 ± 13.3 kg), but there was no difference ($P = 0.2832$) in ADG (0.64 and 0.59 ± 0.055 kg/d) for high and low RFI_c animals; respectively. There were no differences in predicted DMI between RFI_c animals using any n-alkane method ($P > 0.90$). A nonparametric analysis indicated that pre-ranking under confinement does not guarantee ($P < 0.0001$) similar ranking under grazing conditions when using the n-alkane technique to determine forage DMI. The recommendation is that feces are collected twice daily (0700 and 1500 h) to estimate DMI of cattle grazing Coastal bermudagrass pastures stocked at a moderate to low grazing pressure.

Introduction

Recently, the United States beef industry has gone through major changes, due to increases in cost of energy and resultant prices for corn prices, feedstuffs, and fertilizer. As a consequence, beef production from pasture systems is increasing. One of the greatest challenges for beef production under grazing systems is to predict DMI (Lippke, 2002). The profitability of livestock production in grazing systems is related to the efficiency of converting forage into products, the quantity and quality of forage produced, and the ability of the producer to manage the forage (Forbes, 1988). According to Dove and Mayes (2006), measurement of what animals consume, the quality, quantity and grazing behavior is required to study the feeding behavior and nutrition of mammalian herbivores. According to

Lippke (2002), the understandings of factors that influence intake are the only mechanisms that researchers have because there is no practical way to directly measure DMI of grazing animals. The alkane technique has been show to be a viable technique to predict DMI in grazing animals (Mayes and Lamb (1984), Dove and Mayes (1991), Bovolenta et al. (1994), Hameleers and Mayers, (1998), Oliván et al (2007). Knowledge of the amount of forage being consumed by grazing animals is important because it is the major cost input in most animal production systems (Herd et al., 2003).

The objectives of this study were to compare C_{31} and C_{33} n-alkanes in estimating DMI; compare times of fecal collections for estimating DMI; and compare the pre-determined RFI_c and RFI_g using the residual feed intake technique to evaluate whether the original rankings would be sustained on pasture.

Material and Methods

The study was conducted at the Texas AgriLife Research Center in Overton, TX, in humid east Texas (32°16´N 94°59´W, average rainfall 88.9 mm and mean temperature 27.6°C) during the summer of 2008. Purebred Brahman bulls, previously phenotyped under drylot conditions by conventional RFI procedures as either efficient (n = 8, low RFI_c) or inefficient (n =8, high RFI_c) were used. The animals were primarily selected for temperament and arrayed so that they were dispersed by efficiency to pastures. Bulls were stratified into 4 groups with 2 efficient and 2 inefficient bulls. Forage mass was taken in quadrats to ground level. Forage composition was taken to represent diet. Forage composition and mass for all periods are shown in Table 5.1. The animals were weighed every 15 d during all periods of collection. During the experimental period, animals received

an ad libitum commercial mineral supplement (Table 5.2). Preparation of the marker, feeding and feces procedures, grinding, chemical analyses, and alkane determination were described in the previous chapter.

Table 5.1: Chemical composition and available forage in the grazed horizon of the forage during all 3 periods

Items	**Periods**		
	1	2	3
DM[1], %	92.1	92.4	92.5
ADF, %	31.2	27.6	34.4
NDF, %	73.5	71.8	74.0
Lignin, %	3.8	4.5	4.8
EE, %	1.6	1.5	1.5
Ash, %	6.6	5.9	6.5
CP, %	16.6	12.6	15.7
ADIN, %	1.7	1.5	1.5
NDIN,%	7.7	5.5	6.1
SP[2], %	31.7	39.8	37.8
Forage Mass (kg DM/ha)	5794 ± 1016	7044 ± 699	7572 ± 973
Height (cm)	22.7 ± 2.8	24.5 ± 2.7	25.0 ± 4.2

[1] DM calculated after samples were dried for 48 hours in a 60ºC oven
[2] Soluble protein, % of CP

Preparation of the Marker

Corn gluten pellets were prepared at the Texas AgriLife Research Center in Uvalde, TX, and used as the carrier for the n-alkanes. The corn gluten pellets were sieved using a 2-mm sieve to remove fines and 400 ± 1 g was weighed and placed in a paper bag. On the day of preparation, the dosed corn gluten was transferred to a 760-ml Rubbermaid container and placed in an oven at 75ºC for approximately 2 h. Using a 30-ml Minipet Pipettor (VWR, cat # 54848-204), 10 ml of C_{32}-alkane solution was slowly pipetted over the warm corn gluten.

The solution was composed of 7 g of C_{32} (Dotriacontane, Aldrich cat # D22, 310-7) in 350 ml heptane (VWR cat # EM-HX0080-6) and heated on low temperature until a solution was formed. After each set was prepared, warm heptane was used to clean the pipette. After adding the solution over the corn gluten, samples were placed at room temperature to allow the heptane to evaporate for approximately 30 min, and samples were placed in a 75ºC oven for approximately 1 h. Samples were them placed in paper bags and labeled for each trial. One sample of each set was taken for future standard analysis.

Table 5.2: Composition of the mineral supplement

Mineral	**Minimum**	**Maximum**
Calcium, %	11	13
Phosphorus, %	12	-
Salt, %	11	13
Magnesium, %	4	-
Potassium, %	0.5	-
Cooper, ppm	2000	-
Selenium, ppm	26	-
Zinc, ppm	4500	-
Manganese, ppm	2500	-
Iodine, ppm	100	-
Cobalt, ppm	25	-
Vitamin A, i.u./lb	200000	-
Vitamin D, i.u./lb	20000	-
Vitamin E, i.u./lb	100	-

Feeding and Feces Collection Procedures

After one week of adaptation to corn gluten fed via Calan gate units in the pastures, bulls which had been previously trained to eat in Calan gates, were individually fed 400 g of corn gluten two times per day (0700 and 1900 h). Following the adaptation period, bulls were fed corn gluten (400 g) labeled with 200 mg C_{32} n-alkane solution samples twice daily

(0700 and 1900 h) in order to reduce diurnal variation as suggested by Smit et al. (2005). During the first 5 d of the trial, fecal samples were collected twice daily (0700 and 1900 h) and during the following 5 d of the period, fecal samples were collected four times daily (0700, 1100, 1500 and 1900 h). Fecal samples were collected immediately upon defecation or via rectal palpation and placed in zip lock bags. After fecal samples were collected they were placed in a -20 ^{o}C freezer for 24 h. The frozen samples were placed in a 60 ºC oven for 72 h. Forage samples selected to represent the grazed strata were collected daily beginning 2 d prior to the start of dosing of n-alkanes. In order to analyze the difference in n-alkane concentration between different parts of the plant, a leaf/stem separation was performed. There were 3 periods of collections (between June 19th and August 22nd): period 1 (**P1**), period 2 (**P2**), and period 3 (**P3**) interchanged with adaptations periods as shown in Figure 5.1.

Period 1			**Period 2**			**Period 3**	
5 days of adaptation	5 days of collection 4X/day	**Interval** 4 days	5 days of adaptation	5 days of Collection, 4X/day	**Interval** 18 days	5 days of adaptation	5 days of collection 4X/day

Figure 5.1: Collections design of the experimental periods

Chemical Analyzes. All forage and fecal samples were dried at 60 ºC and ground using a cyclone mill fitted with a 1 mm screen, prior to extraction and subsequent gas chromatography. All forage samples were sent to Cumberland Valley Analytical Services for the following analyses: DM was performed in two steps; the first step was according to Goering and Van Soest (1970), and during the second step oven temperature was increase to

105 ºC, according to the National Forage Testing Association (2002). Ash was performed according to AOAC (2002, method 942.05), CP, and non-sequential ADF analyses were performed according to AOAC (2002; method 2001.11 and 973.18*;* respectively). The NDF was determined according to Van Soest et al. (1991). The ether extract (**EE**) was determined by AOAC (2002; method 920.39). The lignin analysis was performed according to Goering and Van Soest (1970) using 72% sulfuric acid with modifications. The corn gluten was ground using a cyclone mill fitted with a 1mm screen.

Alkanes Determination

In order to determine n-alkanes in the fecal and forage samples a gas chromatography system (Agilent 6890N, Santa Clara, CA, USA) with auto sampler and computer program was used. A Supelco Special Order SPB-1, fused silica capillary column, 30 x 0.75mm ID x 1.00 um was used. For each run of analysis, 5 standard samples were included for calibration. The injector was set to add 1.0 ul of sample in a split ratio of 4.3:1 and washed with heptane, pre- and post-injection. The oven temperature was set at 285 ºC and held for 12 min, and the detector heater was set at 320 ºC using a gradient run. Initial temperature was set at 210 ºC, temperature ramped to 285 ºC at 25 ºC/minute and held for eight minutes, then ramped to 310 ºC at 25 ºC/min and held for 2 min. The injector temperature was set at 300 ºC and the detector temperature was set at 320 ºC.

Intake Calculations

Intake calculations were based on a 24-h passage rate of forage and n-alkanes dosed on corn gluten (23% CP, 36% NDF, and 13% ADF). According to Dove and Mays (1996), the intake of small amount of supplement feed that carries the alkane can be disregarded in the intake calculation. Mayes et al. (1986) pointed out the possibility of using C_{31} or C_{33} with adjustment for forage C_{32} in order to estimate DMI of grazing animals. Therefore, four methods of intake calculations were performed: C_{31} and C_{33} with or without adjustments for C_{32} (C_{31}, $C_{33,}$ C_{31_0}, and C_{33_0}, respectively). The first two calculations accounted for C_{32} in the forage intake equation (Eq. 7) while the second two calculations assumed that forage C_{32} was negligible and therefore not accounted for in the forage intake equation (Eq. 8).

DM intake = ((Fecal C_{31}/(Fecal C_{32}-Forage C_{32}) × Dose value)/Forage C_{31})/1000 Eq. [7]

DM intake = (((Fecal C_{31} /Fecal C_{32}) × Dose value)/Forage C_{31})/1000 Eq. [8]

Statistical Analysis

All statistical analyses were conducted with SAS (SAS Inst., Cary, NC, 2008). The PROC GLIMMIX was used for all analyses and the Akaike's Information Criteria (**AIC**) was evaluated when comparing different methods to predict DMI. The least AIC indicates the statistical model with the best goodness-of-fit. The multiple comparisons were performed with LSMeans without any adjustments. The main, fixed factors were treatments and RFI_c. The random factors included period, days within period, animal, and pasture. The variance component was assumed to be the variance-(co)variance matrix.

Evaluation of times and combinations of fecal collections. Times of fecal collections and some combinations were coded as treatments within periods, animal, and pasture. Days of collections were assumed to be replicates. The following treatments were evaluated: 0700 h (**Trt1**), 1100 h (**Trt2**), 1500h (**Trt3**), 1900 h (**Trt4**), average Trt1 and Trt2 (**Trt5**), average Trt1 and Trt3 (**Trt6**), average Trt1 and Trt4 (**Trt7**), average Trt2 and Trt3 (**Trt8**), average Trt2 and Trt4 (**Trt9**), average Trt3 and Trt4 (**Trt10**), and the average of 4 times daily (**Trt11**). This analysis was performed for C_{31} and C_{33} with and without adjustment for forage C_{32} concentration in the forage. The following statistical model was used.

$$DMI = \mu + Trt_i + RFIcj + (Trt \times RFI_c)_{ij} + Period_k + Day_{l(k)}\ Animal_m + Pasture_n + \varepsilon_{ijklmn}$$

where μ is the overall mean and ε is the uncontrolled, random error.

Comparison of Alkane Methods and their Combinations to Determine DMI. Comparisons of four alkane combinations were made (C_{31}, C_{33}, C_{31_0}, and C_{33_0}) to determine DMI of grazing bulls. In this analysis, 6 combinations (Trt) were evaluated: C_{31} with adjustment for forage C_{32} (**TrtA**), C_{33} with adjustment for forage C_{32} (**TrtB**), C_{31} without adjustment for forage C_{32} (**TrtC**), C_{33} without adjustment for forage C_{32} (**TrtD**), the average between TrtA and TrtB (**TrtE**), and the average between TrtC and TrtD (**TrtF**). The following statistical model was used.

$$DMI = \mu + Trt_i + RFIc_j + (Trt \times RFI_c)_{ij} + Period_k + Day_l + Animal_{m(j)} + Pasture_n + Time_{o(l)} + \varepsilon_{ijklmn}$$

Where μ is the overall mean and ε is the uncontrolled, random error.

Comparison of RFI_c and RFI_g. The RFI_c and RFI_g values were computed using a multiple linear regression as shown in Eq. [9] (Arthur et al., 2004). The RFI_g was computed for each alkane method (C_{31}, C_{33}, C_{31_0}, and C_{33_0}) to predict the DMI of the complete feeding period (58 d).

$$\text{Actual DMI} = \text{ADG} + (\text{Mean BW})^{0.75} + \text{RFI} \qquad \text{Eq. [9]}$$

where DMI of the period, kg/d; ADG of the period, kg/d, and RFI is residual feed intake, kg/d.

The comparison of low and high RFI_c groups for forage DMI predicted with each n-alkane method was performed using the PROC GLM (SAS Inst. Inc, Cary, NC). The comparison between previous determinations of efficiency via RFI with a subsequent determination of efficiency via RFI under grazing conditions was performed using a non-parametric analysis, with a 2-way categorical analysis of RFI_c x RFI_g. The frequency of animals in each cell was evaluated. The expected outcome was 8 animals in the high RFI_c and high RFI_g cell and 8 animals in the low RFI_c and low RFI_g cell. The remaining two cells were expected to have zero animals. A one-way variable (HH, HL, LH, and LL in which the first letter represents the ranking of RFI_c and the second letter represents the ranking of RFI_g) was created and a χ^2 test was used to test the expected frequency (8, 0, 0, 8, respectively). Additionally, we performed a linear regression between RFI_c and RFI_g to obtain the correlation coefficient.

Results and Discussion

The total forage DM available to the animals is presented in Table 5.1. The mean DM available to the animals during the study was 6,803 kg/ha. According to Rayburn (1986), in order to maximize DMI pasture organic mass should be at least 2,500 kg/ha. There were 4 periods of collections, but for the first period there was an unpredictable pattern of orts that made it not worthwhile to analyze this data set.

Evaluation of Times of Fecal Collection

There was a difference between Trt5 and Trt10 of DMI predicted by C_{31} (P = 0.0010), C_{33} (P = 0.0001), C_{31_0} (P = 0.0010), and C_{33_0} (P < 0.0001) (Table 5.2). There was no difference (P > 0.05) of predicted DMI for all methods between Trt7 and Trt11. There was a difference in DMI using C_{31}, C_{33}, C_{31_0}, C_{33_0}, mean of C_{31} and C_{33}, and mean of C_{31_0} and C_{33_0}, across days (P = 0.0106, Table 5.3), suggesting several days are needed to estimate DMI. These results agree with Malossini et al. (1996), who worked with mid-lactation cows grazing cool season forages orchard grass (*Dactylis glomera L.*) and kentucky bluegrass (*Poa pratensis L.*) pastures dosed with C_{32} in a capsule and fecal collection performed four times a day. They reported the number of samples collected per day can be decreased to twice daily due to the low variability of the alkane excreted in the feces. Malossini et al. (1994) reported no difference between one fecal sample a day, two, three or four or a composite sample (four samples during the day) to predict DMI in dairy cows grazing cool season red fescue (*Festuca rubra*) and orchard grass as predominant forages. The authors concluded that in order to decrease labor and time one or two samples a day was

adequate to obtain reliable values of predicted DMI. Furthermore, Mann and Stewart (2003) reported a difference ($P < 0.05$) in predicted DMI between morning and afternoon, (8 h interval), in which the morning predicted DMI was underestimated and the afternoon predicted DMI was overestimated compared to actual DMI in yearling bulls fed with kikuyu grass (*Pennisetum clandestinum* Hochst.) in Calan gates and dosed daily with C_{32}. The authors reported that this difference could be eliminated if they had dosed animals twice daily. In this study, marked corn gluten was fed twice daily. One important factor is the time of collection, due to differences in the forage consumed in the morning and in the afternoon. Several authors reported that animals tend to eat more during the afternoon due to increase in nonstructural carbohydrate (Fisher et al., 1999; MacKay et al., 2003), and decrease in fiber components (Mayland et al., 1998), consequently increasing the nutritive value of the forage. This is another reason to collect fecal sample two times a day, morning and afternoon, to balance the diet and the forage intake during the day.

Berry et al. (2000) collected fecal samples 3 times a day (0630, 1330, and 2030 h) from Brown Swiss cows, and concluded that the best time was at 0630 h using C_{33}/C_{32} pairs of alkanes; however, they found no significant difference between times of collections but the variation for the 0630-h collection was less compared to other times of collection. Hameleers and Mayes (1998) found no difference in predicted DMI of dairy cows grazing perennial ryegrass (*Lolium perenne L.*) and white clover (*Trifulium repens L.*) when fecal samples were collected twice a day (am and pm). Mayes et al. (1986) found no variation between times of collection or between days. Those results agree with Oliván et al. (2007), who collected fecal samples three times a day (0830, 1630, and 0030 h) and concluded that

one sample a day was enough to predict DMI. In addition, Reeves et al. (1996) reported no difference in DMI between morning and afternoon estimation of DMI for dairy cows grazing kikuyu grass. Keli et al (2008) reported that sampling once a day is enough to predict DMI in ewes grazing alfalfa (*Medicago sativa L.*) and ryegrass (*Lolium rigidum* Gaudin) treated with C_{32} and C_{36} in paper pellets. There are different conclusions between different studies, although the main differences are in the form in which the n-alkanes are dosed and the number of times a day they are dosed. In this study, in conclusion fecal samples have to be collected twice daily for the most accurate estimation of DMI.

Comparison of alkane methods and their combinations to determine DMI

The best goodness-of-fit (the least AIC) to predict DMI was obtained using C_{31_0} (AIC of 8199.61) followed by C_{33} (AIC of 8661.40). The AIC for C_{31_0} and C_{31} were 10927.92, and 11307.98, respectively. These results agree with those reported in the previous chapter. The average predicted DMI estimated for each method and their combinations are presented in Table 5.4. For all predicted DMI, the C_{31} method with or without adjustment for forage C_{32}, had the highest values. Mayes et al. (1986) working with sheep grazing perennial ryegrass, dosed with alkane shredded papers, and collecting fecal samples every 3 hours, reported that C_{31}/C_{32} pair (C_{31} adjusted for forage C_{32}) underestimated the DMI and the discrepancy sum of squares was less for the C_{33}/C_{32} pair between the actual and estimated DMI. Ferreira et al. (2004) dosed Holstein-Friesian and Barrosa cows with C_{32} and C_{36} using controlled-released capsules and collected fecal samples twice a day (0800, and 2000). They reported no difference between sampling times,

when they calculated DMI using release rates of C_{32} found in their study. However, they assumed a passage rate of 48 h to compute DMI; whereas in this study it was assumed to be 24 h. Keli et al. (2008) predicting DMI in ewes grazing alfalfa and ryegrass treated with C_{32} and C_{36} in paper pellets, found that C_{31} and C_{32} overestimated DMI which was in agreement with the findings in the sense that C_{31} values were greater than C_{33}.

Table 5.3: Comparison of predicted DMI using different n-alkane methods and their combinations with the equation proposed by the NRC (1984) [1]

Treatments [2]	**DMI (kg/d)**	**SEM**
TrtA	9.7[a]	0.52
TrtB	6.4[e]	0.52
TrtC	8.9[b]	0.52
TrtD	5.9[f]	0.52
TrtE	8.0[c]	0.52
TrtF	7.4[d]	0.52
DMI (NRC, 1984)	9.2[b]	

[1] Within a column, means without a common superscript letter differ ($P < 0.05$).

[2] C_{31} with adjustment for forage C_{32} (TrtA), C_{33} with adjustment for forage C_{32} (TrtB), C_{31} without adjustment for C_{32} (TrtC), C_{33} without adjustment for forage C_{32} (TrtD), average between TrtA and TrtB (TrtE), and average between TrtC and TrtD (TrtF).

These DMI estimates for bermudagrass are in agreement with Mayes and Lamb (1984) and Mayes et al. (1986), who have reported an improvement in fecal recovery as alkane chain length increases and consequently better DMI predictions. Ferreira et al. (2004) found no difference between DMI predicted with C_{31}/C_{32} and C_{33}/C_{32} pairs, and both values overpredicted the actual DMI in Holstein-Friesian cows and indigenous Barrosa breed grazing perennial ryegrass, velvetgrass (*Holcus lanatus*) and poaceae (*Bromus sp*) dosed

with C_{32} in controlled-release capsules. In contrast, Smit et al. (2005) reported a variation in DMI of dairy cows grazing perennial ryegrass using C_{31}/C_{32} and C_{33}/C_{32} pairs. In their study, during two consecutive years, C_{31}/C_{32} had greater values than C_{33}/C_{32}, although C_{33}/C_{32} had the least variation. They also concluded that both pairs of alkane overpredicted DMI compared with the energy requirements of the animals. Lambs dosed with C_{32} in capsules, consuming *Lolium perenne*, *Poa* and *Festuca* hay had similar values of predicted DMI with C_{33}/C_{32} and C_{31}/C_{32} compared to actual DMI, and no difference was found when the average of both methods was used (Vulich et al., 1995). The authors reported an overestimation of DMI by 6 % in fecal samples collected directly from the rectum of the animals. On the other hand, Oliván et al. (2007) showed an underestimation of predicted DMI with C_{31}/C_{32} and C_{33}/C_{32} pairs of beef cows consuming alfalfa hay, but these alkane pairs had the greatest deviation in DMI prediction compared to other n-alkanes. Vulich et al. (1991) working with lambs grazing *Lolium perenne*, *Poa* and *Festuca* species dosed with C_{32} capsules, found no variation between predicted DMI with C_{31}/C_{32} and C_{33}/C_{32} pairs or by the average between them, and a high correlation between DMI predict by n-alkanes and the actual DMI.

Using the DMI equation of the NRC (1984), the average estimated DMI for bulls grazing Coastal bermudagrass was 9.21 kg/d (Table 5.5). Comparing this number with the average estimated by C_{31}, C_{33}, C_{31_0}, C_{33_0}, and the average between C_{31} and C_{33} or C_{31_0} and C_{33_0}, the estimated DMI with C_{31} overpredicted DMI predicted by the NRC (1984) equation. There was no difference between DMI predicted by C_{31_0} and the NRC (1984), the other predictions underpredicted DMI.

Table 5.4: Predicted DMI (kg, DM) with different n-alkane methods and different times of fecal collections [1]

	Treatments [2]											
	Trt1	**Trt2**	**Trt3**	**Trt4**	**Trt5**	**Trt6**	**Trt7**	**Trt8**	**Trt9**	**Trt10**	**Trt11**	**SEM**
C_{31}	9.46^{c}	9.41^{c}	9.81^{abc}	10.18^{a}	9.44^{c}	9.64^{bc}	9.81^{ab}	9.61^{bc}	9.78^{abc}	10.00^{ab}	9.71^{abc}	0.795
C_{33}	6.27^{d}	6.30^{cd}	6.54^{abc}	6.78^{a}	6.29^{cd}	6.40^{bcd}	6.53^{abcd}	6.42^{bcd}	6.54^{abc}	6.66^{ab}	6.48^{bcd}	0.528
C_{31_0}	8.68^{c}	8.67^{c}	9.06^{abc}	9.34^{a}	8.68^{c}	8.88^{bc}	9.00^{abc}	8.87^{bc}	9.00^{abc}	9.20^{abc}	8.94^{abc}	0.913
C_{33_0}	5.70^{e}	5.77^{cde}	6.00^{bc}	6.17^{a}	5.74^{e}	5.86^{cde}	5.94^{cde}	5.89^{cde}	5.97^{cd}	6.09^{ab}	5.91^{bcde}	0.406

[1] Within a row, means without a common superscript letter differ ($P < 0.05$)

[2] Using the data from 0700 h (Trt1), 1100 h (Trt2), 1500h (Trt3), or 1900 h (Trt4), or averaging Trt1 and Trt2 (Trt5), Trt1 and Trt3 (Trt6), Trt1 and Trt4 (Trt7), Trt2 and Trt3 (Trt8), Trt2 and Trt4 (Trt9), Trt3 and Trt4 (Trt10), or average of 4 times daily (Trt11)

Herd et al. (1998) reported a better prediction of DMI when the average of C_{31} and C_{33} fecal values were used to predict DMI of Angus cows grazing oat crop (*Avena sativa* L.). Herd et al. (1998) reported a similar value to the DMI predicted by SCA (1990) in this particular situation.

Comparison of RFIc and RFIg

There was a significant difference ($P = 0.0188$) in the mean BW (459 and 409 ± 13.3 kg for high and low RFI_c animals, respectively) at initiation of the experiment. There was no difference ($P = 0.2832$) in ADG between low (0.575 ± 0.055 kg/d) and high (0.661 ± 0.055 kg/d) RFI_c animals during the 58 d period. There were no differences ($P > 0.90$) in predicted DMI of bermudagrass between RFI_c animals using C_{31}, C_{33}, C_{31_0}, C_{33_0}, mean of C_{31} and C_{33}; and C_{31_0} and C_{33_0} (Table 5.5). These results are in agreement with Herd et al. (1998) who found no difference in DMI among low and high RFI Angus cows grazing oats (75%) and ryegrass (25%), dosed with control release capsules contained C_{32} and C_{36}. In addition, Meyer et al. (2008) found that Hereford heifers phenotyped as either low, medium, or high RFI using a GrowSafe system and fed ad libitum unprocessed flakes of square-baled alfalfa-grass mixed hay, had no significant difference in DMI between RFI groups when animals grazed tall fescue pasture. Meyer et al. (2008) calculated DMI using pre- and post-forage yield and a growing degree rate. Furthermore, Dittmar (2007) reported no difference in DMI between previously ranked Brahman heifers as low or high RFI under drylot conditions when they subsequently grazed irrigated tall fescue (*Festuca arundinacea* Schreb) and annual ryegrass.

Table 5.5: Average DMI (kg/d) on bermudagrass predicted with different n-alkane methods for low and high residual feed intake (RFI_c) groups previously determined under confinement conditions

n-alkane method	**RFI_c**		**SEM**	***P*-value**
	Low	High		
C_{31}	9.74[a]	9.68[a]	0.38	0.9008
C_{33}	6.48[a]	6.46[a]	0.29	0.9567
C_{31_0}	8.96[a]	8.91[a]	0.32	0.9082
C_{33_0}	5.92[a]	5.90[a]	0.24	0.9524

Within a row, means with a common superscript letter do not differ ($P < 0.05$)

When C_{31} was used to predict bermudagrass DMI and to calculate RFI_g, 7 bulls kept their rankings, 4 HH and 3 LL (Table 5.6). On the other hand, 9 animals changed their rankings with 4 bulls that were ranked as high RFI_c having switched to a low RFI_g (HL), and 5 bulls that were ranked as low RFI_c having switched to high RFI_g (LH). An identical result was found when C_{31_0} was used to estimate DMI. When C_{33} was used to predict DMI and to calculate RFI_g, 6 bulls kept their rankings, 3 HH and 3 LL. However, 10 bulls changed their rankings, 5 HL and 5 LH. Although C_{33} was different from C_{31} and C_{31_0}, C_{33_0} did not have the same behavior as the C_{33}. When C_{33_0} was used, 7 bulls kept their rankings, 3 HH and 4 LL; however 9 bulls (5 HL and 4 LH) changed their rankings. The results of the one-way analysis to compared the expected proportion of animals (0.5, 0, 0, and 0.5) for HH, HL, LH, and LL combination, respectively, indicated a major re-ranking ($P < 0.0001$), and thus rejecting the null hypothesis that the expected proportion RFI was maintained.

Table 5.6: Frequency of efficient animals determined with residual feed intake (RFI) under confinement (RFI_c) and grazing (RFI_g) conditions

n-alkanes [1]	RFI_g		*P*-values [2]	
	High	Low	H_0: Equal n	H_0: $n_{ij} = E(n_{ij})$
	n = 16			
C_{31}				
RFI_c				
High	4	4	0.3427	<0.0001
Low	5	3		
C_{33}				
RFI_c				
High	3	5	0.2437	<0.0001
Low	5	3		
C_{31_0}				
RFI_c				
High	4	4	0.3427	<0.0001
Low	5	3		
C_{33_0}				
RFI_c				
High	3	5	0.3427	<0.0001
Low	4	4		

[1] n-alkane methods used to predict DMI were based on C_{31} and C_{33} with or without adjustments adjustment for forage C_{32} concentration (C_{31_0} and C_{33_0}; respectively)

[2] H_0: Equal n means same number of animals for each combination of RFI_c and RFI_g, and H_0: $n_{ij} = E(n_{ij})$ means the frequency of animals in the cells HH, HL, LH, and LL (first letter for RFI_c and second letter for RFI_g) were 8, 0, 0, and 8; respectively. Both hypotheses were accessed with the χ^2 test, using the Fisher exact adjustment, and the table probability *P*-value

Figure 5.2 depicts the relationships between RFI_c and RFI_g for each n-alkane method. It confirmed the results obtained with the nonparametric analysis in which there is small or no correlation between RFI_c and RFI_g rankings. After the animals (RFI_g) were ranked there were no differences for BW ($P > 0.6509$) or ADG ($P > 0.3890$) between low and high RFI_g

bulls. For DMI predicted with C_{31}, the BW was 429 and 439 kg for high and low RFI_g, respectively, and the ADG was 0.619 and 0.617 kg/d for high and low RFI_g, respectively. For DMI predicted with C_{33}, the BW was 436 and 432 kg for high and low RFI_g, respectively, and the ADG was 0.590 and 0.646 kg/d for high and low RFI_g, respectively. So, animals can be raked as low and high RFI under dry lot conditions to predict their rankings under grazing conditions.

Implications

The n-alkane technique can be used to estimate bermudagrass DMI on bulls. There was no difference in either DMI (estimated by four n-alkane methods) or ADG in Brahman bulls, previously ranked for RFI under feedlot conditions, grazing Coastal bermudagrass pastures. This suggested that animals ranked under drylot conditions had a different feeding, digestion, and physiological behaviors compared to grazing conditions, and a longer trial could be used to more accurately assess to ADG. Animals should be ranked under grazing conditions. There was a difference in predicted DMI between morning and afternoon fecal collections. Therefore, two collections per day (0700 and 1500 h) are recommended for predicting DMI of Brahman bulls grazing Coastal bermudagrass pastures. A collection in mid-afternoon appears to be a satisfactory compromise between precision and practicality, giving that collection outside regular work hours is hard to schedule.

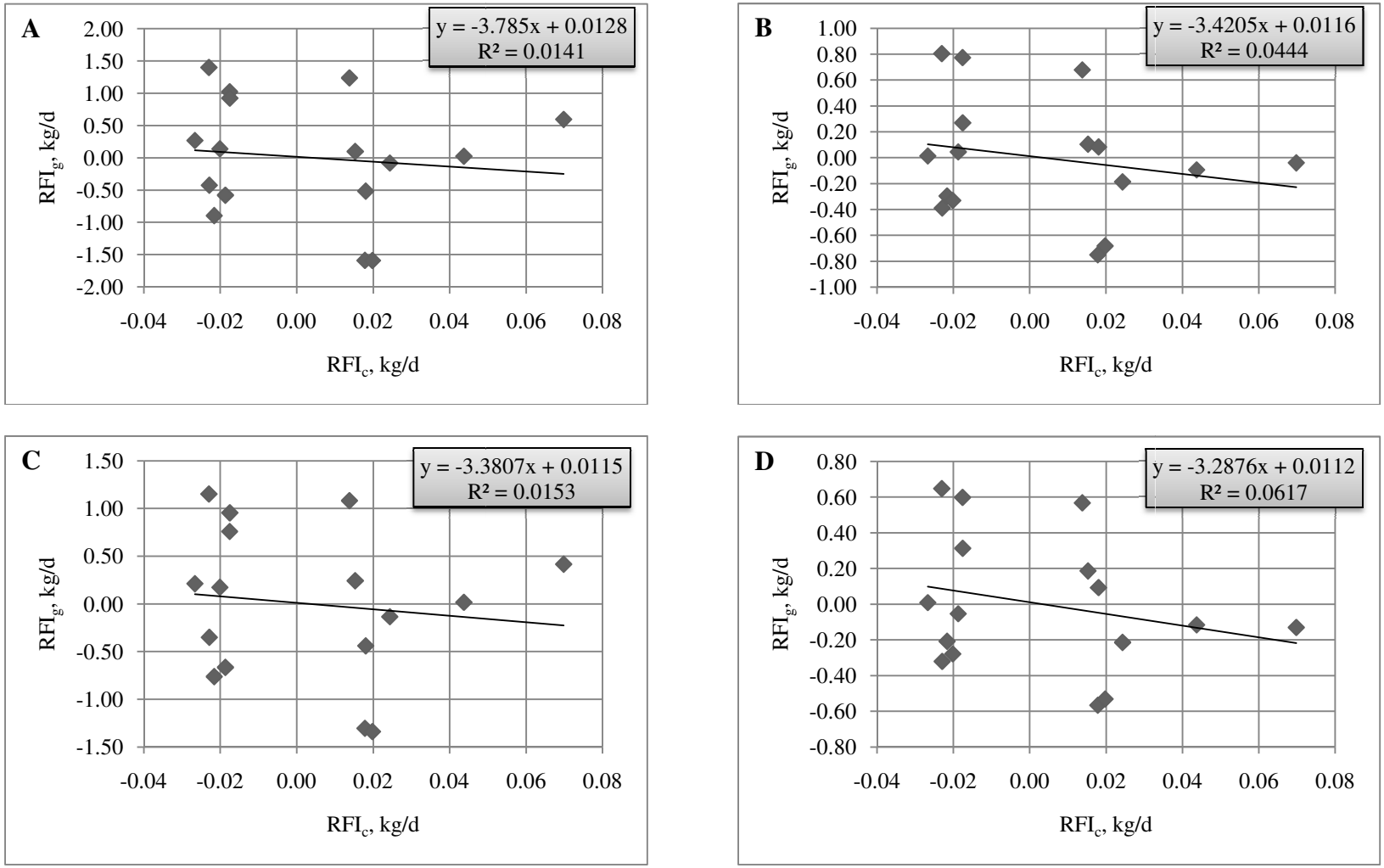

Figure 5.2: Relationship between residual feed intake predicted under confinement (RFI_c) and grazing (RFI_g) conditions using four n-alkane methods to predict DMI (A = C_{31}, B = C_{33}, C = C_{31} no adjustment for forage C_{32}, and D = C_{33} no adjustment for forage C_{32})

LITERATURE CITED

Adesogan, A.T. 2005. Effect of bag type on the apparent digestibility of feeds in ANKOM Daisy ^{II} incubators. Anim. Feed Sci. Technol. 119:333-344.

Allen, M.S., and D.R., Mertens. 1988. Evaluation constraints on fiber digestions by rumen microbes. J. Nutr. 118:261-270.

Allen, V. G., and E. Segarra, 2001. Anti-quality components in forage: Overview, significance, and economic impact. J. Range Manage. 54: 409-412.

Allison, C.D. 1985. Factors affecting forage intake by range ruminants: A review. J. Range. Manage. 38:305-311.

Ammar, H., S. López, J. S. González, and M. J. Ranilla. 2004. Chemical composition and in vitro digestibility of some Spanish browse plant species. J. Sci. Food. Agric. 84: 199 – 204.

AOAC. 2002. Official Methods of Analysis. 17th ed. AOAC, Gaithersberg, MD.

Archer, K.A., and A.M. Decker. 1977. Relationship between fibrous components and *in vitro* dry matter digestibility of autumn-saved grasses. Agron. J. 69:610-612.

Arthur, P. F., J. A. Archer, and R. M. Herd. 2004. Feed intake and efficiency in beef cattle: Overview of recent Australian research and challenges for the future. Austr. J. Exp. Agric. 44:361-369.

Banta, J. P., D. L., Lalman, C.R., Krehbiel, and R.P., Wettemann. 2008. Whole soybean supplementation and cow age class: Effects on intake, digestion, performance, and reproduction of beef cows. J. Anim. Sci. 86:1868-1878.

Barnes R. F. 1968. Use of in vitro rumen fermentation techniques for estimating forage digestibility and intake. Agron. J. 1965 57: 213-216.

Baumann, T.A., G.P. Lardy, J.S. Caton, and V.L. Anderson. 2004. Effect of energy source and ruminally degradable protein addition on performance of lactating beef cows and digestion characteristics of steers. J. Anim. Sci. 82:2667-2678.

Berry, N. R., M.R.L. Scheeder, F. Sutter, T. F. Krober, and M. Kreuzer. 2000. The accuracy of intake estimation based on the use of alkane controlled-release capsules and feces grab sampling in cows. Ann. Zootech. 49:3-13.

Bovolenta S, E., Piasentier, and F. Malossini. 1994. N-alkanes as markers in feeding trials. Pages 29-38 in I.J. Gordon and R. Rubino. Proc. EEC Workshop on Grazing Behaviour of Goats and Sheep. Volume 5. Cahiers Options Méditerranéennes. CIHEAM, Zaragoza.

Burns, J.C. 2008. ASAS Centennial Paper: Utilization of pasture and forages by ruminants: A historical perspective. J. Anim. Sci. 86:3647-3663.

Buxton, D.R., and D.D. Redfearn. 1997. Plant limitations to fiber digestion and utilization. J. Nutr. 127:814S-818S.

Casler, M.D., and H. G. Jung. 2006. Relationships of fibre, lignin, and phenolics to in vitro fibre digestibility in three perennial grasses. Anim. Feed Sci. Technol. 125:151-161.

Casson, T., J. B. Rowe, C. W. Thorn, and D.Harris. 1990. The use of natural n-alkanes in medic and clover as indigestible markers. Proc. Aust. Soc. Anim. Prod. 18: 462-463.

Chicago Board of Trade, 2008. CBOT:
http://www.cbot.com/cbot/pub/page/0,3181,1213,00.html. Accessed August 05, 2009.

Cochran, R. C., and M. L. Galyean. 1995. Measurements of in vivo forage digestion in ruminants. Pages 613-643 in G. C. Fahey, Jr. ed. Forage quality, evaluation, and utilization. AASA, CSSA, and SSSA, Madison, WI.

Coleman, S.W., H. Lippke, and M. Gill. 1999. Estimating the nutritive potential of forages. Pages 647-695 in H. G. Jung, and G. C. Fahey, Jr. (ed.) Nutritional Ecology of Herbivores. Amer. Soc. Anim. Sci., Savoy, IL.

Collins, M., and J.O. Fritz. 2003. Forage Quality. Pages 363 – 390 in R.F., Barnes, C.J. Nelson, M. Collins, and K.J. Moore. Forages: An Introduction to Grassland Agriculture. Volume 1, 6th ed. Blackwell Publishing, Ames IA.

Dhanoa M. S., J. France, L.A. Crompton, R.M. Mauricio, E. Kebreab, J.A.N. Mills, R. Sanderson, J. Dijkstra, and S. Lopez. 2004. Technical note: A proposed method to determine the extent of degradation of a feed in the rumen from the degradation profile

obtained with the in vitro gas production technique using feces as the inoculums. J. Anim. Sci. 82:733–746.

Dittmar III, R. O. 2007. Determining biological sources of variation in residual feed intake in Brahman heifers during confinement and on pasture. M.S. Thesis. Texas A&M University, College Station, TX.

Doane, P.H., P. Schofield, and A.N. Pell. 1997. Neutral detergent fiber disappearance and gas and volatile fatty acid production during the in vitro fermentation of six forages. J Anim. Sci. 75:3342-3352.

Dove, H., and R.W. Mayes. 1991. The use of plant wax alkanes as marker substances in studies of the nutrition of herbage: A Review. Aust. J. Agric. Res. 42:913-952.

Dove, H., and R.W. Mayes. 1996. Plant wax components: A new approach to estimating intake and diet composition in herbivores. American Institute of Nutrition. Manuscript. 13-26.

Dove H., R.W. Mayes, C.S. Lamb, and K.L. Ellis. 2002 Factors influencing the release rate of alkanes from an intra-ruminal, controlled-release device, and the resultant accuracy of intake estimation in sheep. Aust. J. Agric. Res. 53:681:696.

Dove, H., and R.W. Mayes. 2006. Protocol for the analysis of n-alkanes and other plant-wax compounds and for their use as markers for quantifying the nutrient supply of large mammalian herbivores. Nature Protocols 1:1680-1697. http://www.nature.com/nprot/journal/v1/n4/full/nprot.2006.225.html. Accessed June 22, 2009.

Ferreira, L. M. M., M. Oliván, M.A.M. Rodrigues, K. Osoro, H. Dove, and A. Dias-da-Silva. 2004. Estimation of feed intake by cattle using controlled-release capsules containing n-alkanes or chromium sesquioxide. J. Agric. Sci. 142:225-234.

Fisher D. S., H. F. Mayland, and J. C. Burns. 1999. Variation in ruminants' preference for tall fescue hays cut either at sundown or at sunup. J. Anim Sci. 77:762-768.

Forbes, T.D.A. 1988. Researching the plant-animal interface: The investigation of ingestive behavior in grazing animals. J. Anim. Sci.66:2369-2379.

Ford, C.W., I.M. Morrison, and J. R. Wilson. 1979. Temperature effects on lignin, hemicelluloses and cellulose in tropical and temperate grasses. Aust. J. Agric. Res. 30:621-633.

Fox, D. G, C. J. Sniffen, J. D. O'Comor, and P. J. Van Soest. 1992. A net carbohydrate and protein system for evaluating cattle diets: III. Cattle requirements and diet adequacy. J. Anim. Sci. 70:3578-3596.

Fox, D. G., L. O. Tedeschi, T. P. Tylutki, J. B. Russell, M. E. Van Amburgh, L. E. Chase, A. N. Pell, and T. R. Overton. 2004. The Cornell Net Carbohydrate and Protein System model for evaluating herd nutrition and nutrient excretion. Anim. Feed Sci. Technol. 112:29-78.

France J., J. Dijkstra, M.S. Dhanoa, S. Lopez, and A. Bannink. 2000. Estimating the extent of degradation of ruminant feeds from a description of their gas production profiles observed in vitro: derivation of models and other mathematical considerations. British J. Nutr. 83:143–150.

Gao, F., P. Thompson, C. Xiong, and J. P. Miller. 2006. Analyzing multivariate longitudinal data using SAS. Pages 1-9 (Paper 187) in Proceedings of the 31th Annual SAS Users Group International (SUGI) Conference, San Francisco, CA. SAS Institute.

Getachew G., E.J. DePeters, and P. H. Robinson. 2004. In vitro gas production provides effective method for assessing ruminant feeds. California Agriculture 58(1):54-58.

Getachew, G., E.J. DePeters, P.H. Robinson, and J.G. Fadel. 2005. Use of an in vitro rumen gas production technique to evaluate microbial fermentation of ruminant feeds and its impact on fermentation products. Anim. Feed Sci. Technol. 123-124:547-559.

Giráldez F.J., S. López, C.S. Lamb, and R.W. Mayes. 2006. The use of even-chain alkanes sprayed onto herbage as rate of passage markers in goats. Livest Sci. 100:195-202.

Gizzi G.,and D.I. Givens. 2004. Variability in feed composition and its impact on animal production. http://www.fao.org/DOCREP/Article/Agrippa/X9500E03.HTM. Accessed May 13, 2009.

Goering, H. K., and P. J. Van Soest. 1970. Forage fiber analysis: Apparatus, reagents, procedures, and some applications. Pages 1-20 in Agric. Res. Serv. Agric. Handbook. No. 379. USDA, Washington, DC.

Gould, F. W., 1975. Texas Plants - Checklist and Ecological Summary. MP-585/Revised. Texas Agricultural Experiment Station, Dissertation, Texas A&M University, College Station, TX.

Hameleers, A. and R.W. Mayes. 1998. The use of n-alkanes to estimate supplementary grass silage intake in grazing dairy cows. J. Agric. Sci.131:205-209.

Hatfield, P.G., D.C. Clanton, K.M. Eskridge, and D.W. Sanson. 1989. Forage intake by lactating beef cows differing in potential for milk production. J. Anim. Sci. 67:3018-3027.

Hawkings, G.E., G.E. Paar, and J.A. Little. 1964. Composition, intake, digestibility, and prediction of digestibility of coastal bermudagrass hays. J. Dairy Sci. 47:865-870.

Hendricksen R. E., M. M. Reich, R. F. Roberton, D. J. Reid, C. Gazzola, J. A. Rideout, and R. A. Hill. 2002. Estimating the voluntary intake and digestibility of buffel-grass and lucerne hays offered to Brahman-cross cattle using n-alkanes. J. Anim. Sci. 74:567–577.

Herd R.M., J.A. Archer, and P.F. Arthur. 2003. Reducing the cost of beef production through genetic improvement in residual feed intake: Opportunity and challenges to application. J. Anim. Sci. 81:E9-E17.

Herd, R. M., E. C. Richardson, R. S. Hegarty, R. Woodgate, J. A. Archer, and P. F. Arthur. 1998. Pasture intake by high versus low net feed efficient Angus cows. Anim. Prod. Aust. 22: 137-140.

Huhtanen P., A. Seppa la, M. Ots, S. Ahvenjarvi, and M. Rinne. 2008. In vitro gas production profiles to estimate extent and effective first-order rate of neutral detergent fiber digestion in the rumen. J. Anim. Sci. 86:651–659.

Hungate, R.E., 1966. The Rumen and its Microbes. Academic Press, New York, NY.

Iantcheva N., H. Steingass, N. Todorov, and D. Pavlov. 1999. A comparison of in vitro rumen fluid and enzymatic methods to predict digestibility and energy value of grass and alfalfa hay. Anim. Feed Sci. Technol. 81:333-344.

Juarez Lagunes, F.I., D.G. Fox, R.W. Blake, and A.N. Pell. 1999. Evaluation of tropical grasses for milk production by dual-purpose cows in tropical Mexico. J. Dairy Sci. 82:2136-2145.

Jung, H. G., and J. Ralph. 1990. Phenolic-carbohydrate complexes in plant cell walls and their effect on lignocellulose utilization. Pages 173-182 in D. E. Akin, L. G. Ljungdahl, J. R. Wilson, and P. J. Harris. Microbial and Plant Opportunities to Improve Lignocellulose Utilization by Ruminants. ed. Elsevier, New York, NY.

Keli, A., D. Andueza, A. de Vega, and J.A. Guada. 2008. Validation of the n-alkane and NIRS techniques to estimate intake, digestibility and diet composition in sheep fed mixed lucerne: Ryegrass diets. Livestock Sci. 119: 42–54

Kloppenburg, P.B., H.E. Kiesling, R.E. Kirksey, and G.B. Donart. 1995. Forage quality, intake, and digestibility of year-long pastures for steers. J. Range Manage. 48:542-548.

Krishanmoorthy U, H. Soller, H. Steingass, and K.H. Menke. 1995. Energy and protein evaluation of tropical feedstuffs for whole tract and ruminal digestion by chemical analyses and rumen inoculum studies in vitro. Anim. Feed Sci. Technol. 52: 177-188.

Krishanmoorthy U., C. Rymer, and P.H. Robinson. 2005. The in vitro gas production technique: Limitations and opportunities. Editorial/ Anim. Feed Sci. Technol. 123-124:1-7.

Krstic L.N., L.S. Merkulov, J. Z. Lukovic, and P. P. Boza. 2008. Histological components of *Trifolium L.* species related to digestive quality of forage. Euphytica. 160:277-286.

Laredo M.A., G.D. Simpson, D.J. Minson, and C.G. Orpin. 1991. The potential for using n-alkanes in tropical forages as a marker for the determination of dry matter by grazing ruminants. J. Agric. Sci. 177:355-361.

Lippke, H. 2002. Forage & grazing lands: Estimation of forage intake by ruminants on pasture. Crop. Sci.42:869-872.

Makkar, H. P. S. 2004. Recent advances in the in vitro gas method for evaluation of nutritional quality of feed resources. Pages 55–88 in Animal Production and Health Div. FAO. Food and Agriculture Organization of United Nations, Rome.

Malossini F., S. Bovolenta, E. Piasentier, and M. Valentinotti. 1994. Variability of n-alkane content in a natural pasture and in feces of grazing dairy cows. Anim. Feed Sci. Technol. 50:113-122.

Mallosini F., S. Bovolenta, E. Piasentier, C. Piras, and F. Martilloti. 1996. Comparison of n-alkanes and chromium oxide methods for estimating herbage intake by grazing dairy cows. Anim. Feed Sci. Technol. 61:155-165.

Mandebvu, P., J. W. West, G. M. Hill, R. N. Gates, R. D. Hatfield, B. G. Mullinix, A. H. Parks,, and A. B. Caudle. 1999. Comparison of Tifton 85 and Coastal bermudagrasses for yield, nutrient traits, intake, and digestion by growing beef steers. J. Anim. Sci. 77:1572–1586.

Mann J., and P.G. Stewart. 2003. Kikuyu (*Pennisetum clandestinum*) intake determined by alkanes administered in a xantham gum suspension. South African J. Anim. Sci. 33:27-31.

Marten, G. C., and R. F. Barnes. 1980. Prediction of energy digestibility of forages with in vitro rumen fermentation and fungal enzymes systems. Pages 61-71 in Standardization of Analytical Methodology for Feeds. Pidgen, W.J., C.C. Balch, and M. Graham, ed. International Development Research Center, Ottawa, Canada.

Mayes, R.W., and C.S. Lamb. 1984. The possible use of n-alkanes in herbage as indigestible fecal markers. Proc. Nutr. Soc. 43:39A.

Mayes, R.W., C.S. Lamb, and P.M. Colgrove. 1986. The use of dosed and herbage n-alkanes as marker for the determination of herbage intake. J. Agric. Sci., Cam. 107:161-170.

Mayland, H.F., G.E. Shewmaker, J.C. Burns, and D.S. Fisher. 1998. Morning and evening harvest effects on animal performance. Pages 26-30 in Proceedings, 1998 California Alfalfa Symposium, 3-4 Dec. 1998, Reno, NV, UC Cooperative Extension, University of California, Davis.

McBee, R.H. 1953. Manometric method for the evaluation of microbial activity in the rumen with application to utilization of cellulose and hemicelluloses. Appl. Microbiol. 1:106-110.

McDonald, P., R. A. Edvards, J. F. D. Greenhalgh, and C. A. Morgan. 1995. Animal Nutrition. 5th ed. Longman Scientific & Technical, New York, NY.

MacKay L.C., H.F. Mayland, and W.P. MacKay. 2003. Horse preference for alfalfa-grass hay harvest in the afternoon or morning. Not paginated in Proc. Western Section, Amer. Soc. Anim. Sci. Vol. 54.

Menke, K.H., L. Raab., A. Salewski, H. Steingass, D. Fritz, and W. Schneider. 1979. The estimation of the digestibility and metabolizable energy content of ruminant feedstuffs from the gas production when they are incubated with rumen liquor *in vitro.* J. Agri. Sci. 93:217-222.

Menke, K. H., and H. Steingass. 1988. Estimation of the energetic feed value obtained from chemical analysis and in vitro gas production using rumen fluid. Anim. Res. Dev. 28: 7-55.

Meyer M. W., and R. D. Brown, 1985. Seasonal trends in the chemical composition of ten range plants in South Texas. J. Range Manage. 38:154-157.

Meyer, J.H.F., and R.I. Mackie. 1986. Microbiological evaluation of the intraruminal in sacculus digestion technique. Appl. Environ. Microbiol. 51:622-629.

Meyer A.M., M.S. Kerley, and R.L. Kallenbach. 2008. The effect of residual feed intake classification on forage intake by grazing beef cows. J. Anim. Sci. 86:2670-2679.

Miller, J.R., and N.T. Hobbs. 1994. Effect of forage hydration on lag time during in vitro digestion of meadow hay. Grass and Forage Sci. 49:107-110.

Minson, D.J. 1990. Forage in Ruminant Nutrition. Academic Press, San Diego, CA.

Molina D.O., I. Matamoros, and A.N. Pell. 2004. Accuracy of estimates of herbage intake of lactating cows using alkanes: Comparison of two types of capsules. Anim. Feed Sci. Technol. 114:241-260.

Molina, D.O., I. Matamoros, Z. Almeida, L.O. Tedeschi, and A.N. Pell. 2004. Evaluation of the dry matter intake predictions of the Cornell Net Carbohydrate and Protein System with Holstein and dual-purpose lactating cattle in the tropics. Anim. Feed Sci. Technol. 114:261-278.

Moore, K.J., and H. G. Jung. 2001. Lignin and fiber digestion. J. Range Manage. 54:420-430.

Moran J. 2005. What is in feeds? Pages 27-39 in Tropical Dairy Farming: Feeding Management for Small Holder Dairy Farmer in the Humid Tropics. Landlinks Press, Collinwood, Australia.

Moreira F.B., I.N. Prado, U.Cecato, F.Y. Wada, and I.Y Mizubuti. 2004. Forage evaluation, chemical composition, and in vitro digestibility of continuously grazed star grass. Anim. Feed Sci. Technol. 113:239-249.

Mota, M., R. Rodríguez, E. Solanas, and M. Fondevila. 2005 Evaluation of four tropical browse legumes as nitrogen source: Comparison of in vitro gas production with other methods to determine N degradability. Anim. Feed Sci. Technol. 123-124:341- 350.

Nelsen, T., T.C. Cartwright, A.K. Angirasa, and F.M. Rouquette, Jr. 1982. Simulated effect of calving season and winter hay feeding level on cow herd productivity. J. Anim. Sci. 54:29-34.

NRC. 1984. Nutrient Requirements of Beef Cattle. 6th Rev. ed. National Academy Press, Washington, DC.

NRC. 2000. Nutrient Requirements of Beef Cattle. 7th ed. National Academy Press, Washington, DC.

Oliván M., L.M.M. Ferreira, R. Celaya, and K.Osoro. 2007. Accuracy of the n-alkane technique for intake estimates in beef cattle using different sampling procedures and feeding levels. Livest. Sci. 106:28-40.

Oliveira A.F.M., and A. Salatino. 2000. Major constituents of foliar epicuticular waxes of species from caatinga and cerrado, Z. Naturforsch. 55: 688–692.

Ovenell, K. H., K. S. Lusby, G. W. Horn, and R. W. McNew. 1991. Effects of lactational status on forage intake, digestibility, and particulate passage rate of beef cows supplemented with soybean meal, wheat middlings, and corn and soybean meal. J. Anim. Sci. 69:2617–2623.

Pacheco, M.E., R.D. Brown, and R.L. Bingham. 1982. Nutritive value and intake of Kleberg Blue-stem by beef cattle. J. Range Manage. 36(2):222-224.

Pell, A.N., and P. Schofield. 1993. Computerized monitoring of gas production to measure forage digestion in vitro. J. Dairy Sci. 76:1063–1073.

Pitman, W.D., and E.C. Holt. 1982. Environmental relationships with forage quality of warm-season perennial grasses. Crop Sci. 22: 1012-1016.

Quanbek, K., and R. J. Johson. 2009. Livestock, Dairy, and Poultry Outlook: Livestock inventories respond to decreased demand. A report from the Economic Research Service. Pages 1-29. http://www.ers.usda.gov/Publications/LDP/2009/08Aug/ldpm182.pdf. Accessed September 25, 2009

Rayburn, E.B.1986. Quantitative Aspects of Pasture Management: Seneca Trail RC&D Technical Manual. Seneca Trail RC&D, Franklinville, NY.

Reeves, M., W.J. Fulkerson, R.C. Kellaway, and H. Dove. 1996. A comparison of three techniques to determine the herbage intake of dairy cows grazing kikuyu (*Pennisetium clandestinum*) pasture. Aust J. Exp Agric. 36:23-30.

Reynoso-Campos, O., D. G. Fox, R. W. Blake, M. C. Barry, L. O. Tedeschi, C. F. Nicholson, H. M. Kaiser, and P. A. Oltenacu. 2004. Predicting nutritional requirements and lactation performance of dual-purpose cows using a dynamic model. Agric. Syst. 80:67-83.

Robinson P.H., D.I. Givens, and G. Getachew. 2004. Evaluation of NRC, UC Davis and ADAS approaches to estimate the metabolizable energy values of feeds at maintenance energy intake from equations utilizing chemical assays and in vitro determinations. Anim. Feed Sci. Technol. 114:75-90.

Rouquette F. M., Jr., L. A. Redmon, G. E. Aiken, G. M. Hill, L. E. Sollenberger, and J. Andrae. 2009. ASAS Centennial Paper: Future needs of research and extension in forage utilization
J. Anim. Sci. 87: 438-446.

Rymer, C., J.A. Huntington, B.A. Williams, and D.I. Givens. 2005. In vitro cumulative gas production techniques: History, methodological considerations and challenges. J. Ani. Feed Sci. Technol. 123-123:9-30.

Schofield, P., R.E. Pitt, and A.N. Pell. 1994. Kinetics of fiber digestion from in vitro gas production. J. Anim. Sci. 72:2980-2991.

Schofield, P., and A.N. Pell. 1995. Measurement and kinetic analysis of the neutral detergent-soluble carbohydrate fraction of legumes and grasses. J. Anim. Sci. 73:3455-3463.

Smit, H.J., H.Z. Taweel, B.M., Tas, S. Tamminga, and A. Elgersma. 2005. Comparison of techniques for estimating herbage intake of grazing dairy cows. J. Dairy Sci. 88:1827-1836.

Smith, L.L., and J.R. Strickland. 2007. Improved GC/MS method for quantification of n-alkanes in plant and fecal material. J. Agric. Food Chem. 55:7301-7307.

Sniffen, C. J., J. D. O'Connor, P. J. Van Soest, D. G. Fox, and J. B. Russell. 1992. A net carbohydrate and protein system for evaluating cattle diets: II. Carbohydrate and protein availability. J. Anim. Sci. 70:3562–3577.

Sowell, B.F., Bowman, J.G.P., E.E. Grings, and M.D. MacNeil. 2003. Liquid supplement and forage intake by range beef cows. J. Anim. Sci. 81:294-303.

Sprinkle J.E. 1996. Matching forage resources with cow herd supplementation. Arizona Cooperative Extension. Publication number 195023. Pages 1-10. http://ag.arizona.edu/pubs/animal/az9523.pdf.

Tedeschi, L. O., D. G. Fox, and P. H. Doane. 2005. Evaluation of the tabular feed energy and protein undegradability values of the National Research Council nutrient requirements of beef cattle. Prof. Anim. Scientist. 21:403-415.

Tedeschi, L. O. 2006. Assessment of the adequacy of mathematical models. Agric. Sys. 89: 225-247.

Tedeschi, L. O., P. Schofield, and A. N. Pell. 2008a. Determining feed quality for ruminants using in vitro gas production technique. 1. Building an anaerobic fermentation chamber. Beef Cattle Research in Texas. Texas A&M University, College Station, TX (in press).

Tedeschi, L. O., P. Schofield, and A. N. Pell. 2008b. Determining feed quality for ruminants using in vitro gas production technique. 2. Evaluating different models to assess gas production measurements. Beef Cattle Research in Texas. Texas A&M University, College Station, TX (in press).

Tedeschi L.O, P.J. Kononoff, K. Karges, and M.L. Gibson. 2009. Effects of chemical composition variation on the dynamics of ruminal fermentation and biological value of corn milling (co)products. J. Dairy Sci. 92, 401-413.

Tilley, J.M.A., and R.A. Terry. 1963. A two-stage technique for the digestion of forage crops. J. British Grassland Soc. 18:104-111.

Traxler, M. J., D. G. Fox, P. J. Van Soest, A. N. Pell, C. E. Lascano, D. P. D. Lanna, J. E. Moore, R. P. Lana, M. Vélez, and A. Flores. 1998. Predicting forage indigestible NDF from lignin concentration. J. Anim. Sci. 76:1469-1480.

United States Department of Agriculture. 2007 Census Of Agriculture - United States Data. http://www.agcensus.usda.gov/Publications/2007/Full_Report/Volume_1,_Chapter_1_US/st99_1_012_013.pdf. Accessed on September 09, 2009.

United States Department of Agriculture. 2008 Agricultural Outlook: Statistical Indicators. Table 29. http://www.ers.usda.gov/publications/agoutlook/aotables/2008/11Nov. Accessed on September 09, 2009.

Valiente, O.L, P. Delgado, A. de Vega, and J.A. Guada. 2003. Validation of the n-alkane technique to estimate intake, digestibility, and diet composition in sheep consuming mixed grain: Roughage diets. Aust. of J. Agric. Res. 54:693-702.

Van Keuren, R. W., and W. W. Heinemann. 1962. Study of a nylon bag technique for in vivo estimation of forage digestibility. J. Anim. Sci. 21:340.

Van Soest, P. J. 1973. The uniformity and nutritive availability of cellulose. Fed. Proc. 32: 1804.

Van Soest, P.J. 1994. Nutritional Ecology of the Ruminant. 2nd ed. Cornell University Press, Ithaca, NY.

Van Soest, P.J., J.B. Robertson, and B.A. Lewis. 1991. Methods for dietary fiber, neutral detergent fiber and non-starch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74:3583–3597.

Vieira, R. A. M., L. O. Tedeschi, and A. Cannas. 2008a. A generalized compartmental model to estimate the fibre mass in the ruminoreticulum. 1. Estimating parameters of digestion. J. Theor. Biol. 255:345-356.

Vieira, R. A. M., L. O. Tedeschi, and A. Cannas. 2008b. A generalized compartmental model to estimate the fibre mass in the ruminoreticulum. 2. Integrating digestion and passage. J. Theor. Biol. 255:357-368.

Vulich, S.A., E.G. O'Riordan, and J.P. Hanrahan. 1991. Use of n-alkanes for the estimation of herbage intake in sheep: Accuracy and precision of the estimates. 1991. J. of Agric. Sci, Camb. 116:319-323.

Vulich, S.A., J.P. Hanrahan, and B.A. Crowley. 1995. Modification of the analytical procedures for the determination of herbage and faecal n-alkanes used in the estimation of herbage intake. J. Agric. Sci., Camb 124:71-77.

Weiss, W.P., H.R. Conrad, and N.R. St-Pierre. 1992. A theoretically-based model for predicting total digestible nutrient values of forages and concentrates. Anim. Feed Sci. Technol., 39: 95-110.

Welch J. G. and A. P. Hooper. 1988. Ingestion of feed and water. Pages 108–116 in The Ruminant Animal: Digestive Physiology and Nutrition. D. C. Church ed. Prentice-Hall, Englewood Cliffs, N.J.

Wilson, J.R., and P.W. Hattersley. 1983. In vitro digestion of bundle sheath cells in rumen fluid and its relation to the suberized lamella and C4 photosynthetic type in Panicum species. Grass Forage Sci. 38: 219-223.

Winterholler S.J., D.L. Lalman, M.D. Hudson, and C.L. Goad. 2009. Supplemental energy and extruded-expelled cottonseed meal as a supplemental protein source for beef cows consuming low-quality forage. J. Anim. Sci. 87:3003-3012.

Yayneshet T., L.O. Eik, and S.R. Moe. 2009. Seasonal variations in the chemical composition and dry matter degradability of exclosure forages in the semi-arid region of northern Ethiopia. Anim. Feed Sci. Technol. 148:12-33.

Yu, J., J. Norwine, R. Bingham, and C. Tebaldi. 2006. Potential climatic deterioration in semiarid subtropical South Texas. Geography on line.

http://www.siue.edu/GEOGRAPHY/ONLINE/gov6n2.html. Accessed Oct. 9, 2009.

VITA

Name: André De Stefani Aguiar

Address: University of Florida
Agronomy Department
304 Newell Hall, PO Box 110500
Gainesville, FL 32611-0500

Email Address: e4aguiar@gmail.com

Education: B.S., Animal Science, Faculdades Associadas de Uberaba, 2007

M.S., Animal Science, Texas A&M University, 2010

Ph.D., Agronomy, University of Florida, present